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Camellia sinensis constituents:
A Review of Oral Cancer Prevention

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Abstract

Historically, Camellia sinensis (tea) is a plant that has been known to contain antioxidants. Antioxidants such as catechins have been demonstrated to be chemopreventive agents. This review aims to summarize recent findings on the anticancer properties of tea, and its constituents. Since tea is taken orally, and one of the easiest entrances into the human body for microbes is through the oral cavity, this review will focus mainly on oral cancer.

Through animal and epidemiological studies, the main active ingredient responsible for the anticancer properties of tea has been determined to be the catechin (-)-epigallocatechin gallate (EGCG). Tea constituents were analyzed through the use of HPLC and confirmed by comparison to authentic standards and mass spectrometry.

The results obtained from some studies conflicted with earlier notions that tea catechins act as antioxidants, inhibiting cancer cells. They discovered the catechins to have a pro-oxidant effect, generating reactive oxygen species, such as H$_2$O$_2$. Methods of cancer inhibition were also explored, including cell cycle arrest at certain checkpoints and induction of apoptosis, the active process of cell death.

Results from a current study were also examined. Anti-viral and anti-bacterial effects of green and white teas were determined using the plaque method and the Kirby-Bauer, disk-diffusion technique. Results indicated the power of whole tea and tea constituents alongside toothpaste and gum. More than 99% inactivation of viruses was obtained in ten minutes using Tom’s of Maine toothpaste with white tea, whereas infusion of tea into chewing gum yielded over 90% inactivation. Furthermore, distinct zones of inhibition were present for toothpastes and gum treated with tea than for the oral agents by themselves. The future of the research was also briefly discussed.

Although many studies have shown beneficial properties of Camellia sinensis, much more epidemiological research remains to be conducted in order to observe the effects on human cancer cells.

KEY WORDS: Camellia sinensis · tea · oral cancer · EGCG · pro-oxidant
**Introduction**

*History of Tea.* Tea is “the most consumed beverage in the world, second only to water” (Mukhtar et al., 2000). The first mention of tea was recorded in an ancient Chinese wordbook in 350 B.C. Until the 6th century, tea as a beverage remained a tradition in China. Its uses then spread to Japan, Indonesia, Holland, and England (Weisburger, 1997). The components of tea include catechins, polyphenols, and other antioxidants. These components are termed “nonnutrient”, or chemopreventive agents that may reduce the risk of cancer (Dreosti, 1996; Mukhtar et al., 2000; Weisburg et al., 2004). Past studies have demonstrated anticarcinogenic activity of tea in cancers of the skin, lungs, gastro-intestinal tract, colon and urinary bladder (Khafif et al., 1998; Cooper et al., 2005).

*Oral cancer in developing and industrialized countries.* Oral cancer has been prominent in third world countries such as India, Sri Lanka, and Vietnam (Li et al., 1999). Compared to industrialized nations, these developing countries have lower occurrences of common cancers, but have more than half the global cancer burden. Mortality rates per 100,000 individuals for common cancers, including that of the lungs, breasts, and cervix, are 54.4 for males and 36.4 for females in Thailand, 77.3 and 78.2 in Mexico. In industrialized countries these figures are much higher, including rates over 150 for males and over 100 for females. Poor communication and transportation, as well as inadequate finances and training of health professionals in these countries lead to difficulties in performing cancer research (Magrath et al., 1993). The Journal of the National Cancer Institute (1993) reported that oral cancer especially predominates in Asia as a result of chewing “pan”, locally manufactured cigarettes. High rates of oral cancer are also documented in Indian men and in Papua New Guinea, where oral cancer is the most frequent cancer in men and the third most frequent in women. The high oral cancer rates are owed to the chewing of tobacco (Magrath et al., 1993). Although developing nations hold an increasing cancer burden, oral cancer has also increased in America, Japan, Germany and China (Li et al., 1999). Factors such as environment, lifestyle, diet and increase in tobacco consumption are important determinants of cancer patterns in both types of nations (Magrath et al., 1993; Weisburger, 1997).

*Tea Varieties.* There are various types of teas, some of which have greater roles in anticarcinogenic activity than others (Santana-Rios et al., 2001). The four main types of teas include green, black, oolong, and white. These varieties all come from the same plant: *Camellia Sinensis* (Ahmad, et al., 1997; Yang, et al., 1999; Mukhtar, et al., 2000; Lambert, et al., 2003; Cooper, et al., 2005).
Green tea is first pan-fried and steamed, then rolled, shaped, and finally dried. The major polyphenol components of green tea include catechins (-)-epigallocatechin gallate (EGCG), epicatechin (EC), gallocatechin (GC), gallocatechin gallate (GCG), epigallocatechin (EGC), and epicatechin gallate (ECG) (Dreosti, 1996; Cooper et al., 2005).

Black tea, on the contrary, is crushed before being pan-fried and dried. The crushing allows the leaves to go through an oxidation, whereupon the tea’s characteristic theaflavins and thearubigins are generated, totaling 2-6% and more than 20%, respectively (Lambert et al., 2003). Since these components are only found in black tea, this tea has a lower catechin level compared to green tea (Dreosti, 1996). The catechins only make up 3-10% dry weight of a cup of brewed black tea, compared to 30% in green tea (Weisburger, 1997; Li et al., 1999; Lambert et al., 2003).

Oolong tea preparation is similar to black tea, with the exception that the oxidation is brief. White tea, unlike the other teas that go through a primary withering stage, is simply steamed and dried. White tea, therefore, is the most natural and least processed of the tea varieties (Mukhtar et al., 2000; Santana-Rios et al., 2001; Lambert et al., 2003; Schiffenbauer, 2005).

*Tea and cancer.* Tea has been demonstrated to inhibit cancer cells in a variety of methods. Khafif, et al., (1998) were first to publish a study on EGCG affects on the cell cycle. Results indicated that EGCG blocked cells in the G1 phase of the cell cycle. Lambert, et al., (2003) showed that EGCG blocks the G1 phase, as well as the G0 phase. The G2 and M phases of the cycle have also been shown to go through phase arrest upon exposure to EGCG in human lung cancer cells (Cooper et al., 2005). Furthermore, EGCG was determined to induce apoptosis, the active process of cell death, of cancer cells. This was conducted by blocking DNA transcription in the genes of cancer cell lines, including activator protein 1 and nuclear factor κB (Lambert et al., 2003; Cooper et al., 2005). The inactivation of DNA was also observed through the inhibition of topoisomerase I by EGCG in human colon cancer cells. This process requires less doses of EGCG (10-17 μmol/L) than the dose used to inhibit cell growth (10-90 μmol/L) (Lambert et al., 2003). Likewise, research conducted by Yang, et al., (1998) highlighted the growth inhibitory activities of EGCG and EGC in human lung tumor cells lines H661 and H1299. An assay of [3H] thymidine was added to the cancer cells infused with tea polyphenols. The cells that underwent apoptosis were analyzed under a fluorescence microscope, or by flow cytometry. The catechin ECG was found to have less inhibiting activity than EGCG toward these cell lines, and the catechin EC was found to be the least active.

black tea, most likely because of the higher EGCG content. Although Lambert, et al., (2003) agree with this study in that EGCG induces H$_2$O$_2$ as pro-oxidants, they also highlighted EGCG as inhibitors of H$_2$O$_2$, as part of the catechin’s antioxidant activity. Carcinoma of the tongue was found to be most sensitive to EGCG exposure, whereas carcinoma of the salivary gland was least sensitive. Morphological changes, including cellular flattening and irregularity, of these carcinomas were visible in experimental procedures (Weisburg et al., 2004). Suggested explanations for these results identified EGCG as scavengers for free radicals and maintainers of cell survival (Langley-Evans, 2000; Mukhtar et al., 2000; Weisburg et al., 2004). Conversely, high concentrations of EGCG lead to cellular damage and death (Weisburg et al., 2004).

**Green vs. white teas.** Although there has been research performed on white tea, the benefits of this tea variety are in the process of being exposed to the public. One study performed by Santana-Rios, et al, (2001) has compared the antimutagenic activities of green and white teas. A concentration of 2 g. tea leaves per 100 ml of water was brewed for 10 min. Exocita White Tea and Premium Green Tea were tested against 0.01 ml of the heterocyclic amine IQ in 0.2 ml of *Salmonella typhimurium* strain TA98 in the presence of 0.2 ml rat liver S9. Results indicated that Exotica white tea was significantly more effective at inhibiting the mutagen than Premium green tea. Approximately 1,000 green tea revertants per plate were counted after 6 min. of brewing time, compared to 200 white tea revertants per plate. To determine the difference between the properties of green and white teas, high performance liquid chromatography (HPLC) and UV spectral analyses were performed to identify the major compounds present in the teas (Santana-Rios et al., 2001). EGCG was found to be present at equal levels in both teas. Although nine of the major compounds in green tea were also found in white tea, the study found that the differences might be in the levels of the compounds, with white tea acting in combination with other minor unidentified compounds.

**Current Research.** The present study maintains that tea catechins work better individually rather than in combination at inhibiting pathogenic viruses and bacteria, and that white tea is a more effective anti-microbial agent than green, black, or oolong teas. The anti-viral effects of the teas were determined using the plaque technique, and the anti-bacterial effects were examined via the Kirby-Bauer method on *Streptococcus mutans*, which is the main bacterium responsible for plaque and cavity development. The constituents of these teas, including EGCG, EGC, ECG, EC, and CAT ((+)-Catechin), were analyzed using HPLC and their anti-viral and anti-bacterial effects were compared to whole tea solutions.
Methods

To examine the different responses of malignant and normal cells upon addition of black and green teas, as well as the main tea catechin EGCG, a study by Weisburg, et al., (2004) was performed with cells from the human oral cavity. These cells included cultures of normal gingival (GN56) fibroblasts, tongue squamous carcinoma (CAL27) cells, salivary gland carcinoma (HSG1) cells, squamous carcinoma (HSC-2) cells derived from the floor of the oral cavity, gingival epithelial (S-G) cells, and normal gingival (HGF-1) fibroblasts. 1-1.2 x 10^4 of each cell type was cultured and cytotoxicity was assessed using 0.2 ml of the neutral red assay, with an exposure period of 72 hours. Cells were examined microscopically. The viability of the cells was tested based on uptake and accumulation of the dye. Measurement of H_2O_2 was performed by the FOX (ferrous ion oxidation xylenol orange) method. A sample containing 90 µl of exposure medium amended with EGCG, green tea polyphenolic extract, and black tea polyphenolic extract was added to 0.9 ml of FOX reagent, followed by centrifugation and absorbance readings at 580 nm. All of the experiments were conducted at least three times. The midpoint cytotoxicity, or the concentration of the agents necessary to reduce the neutral red dye by 50%, was assessed using linear regression analyses. A one-way analysis of variance (ANOVA) was used to evaluate significant differences in data.

To further recognize changes in cancer cells in response to tea catechins, the process of apoptosis was monitored. To identify apoptosis cells were stained and analyzed via flow cytometry. Ahmad, et al., (1997) observed apoptosis in the human epidermoid carcinoma cell line A431 via DNA fragmentation. The cells were grown at a density of 1 x 10^6 cells and treated with 40, 80, and 160 µg/ml of green tea polyphenol, as well as with 40 µg/ml of individual constituents, including EGCG, ECG, EGC, and EC for 48 hours. The cells were then stained with 10 µM of SYTO 13, a stain indicative of apoptosis. This procedure allowed the cells that underwent apoptosis to be analyzed using flow cytometry.

Since the induction of apoptosis may be controlled by cell cycle regulation, experiments were also performed to examine the effect of EGCG on the cell cycle. Ahmad, et al., (1997) arrested the A431 cells in the G_0 phase by starving them of serum for 36 hours. They were then treated with 40 and 80 µg/ml of EGCG and analyzed by flow cytometry.

To establish whether green tea polyphenol, black tea polyphenol, or a mixed tea solution containing black and green tea polyphenol function better at reducing oral carcinogenesis, Li, et al., (1999) experimented with golden Syrian hamsters. These hamsters were chosen because of
their buccal pouch, whose development closely resembles that of precancerous lesions in the human oral cavity. The sample included 138 hamsters, which were separated into four groups of 32 for the positive control, green tea polyphenol, black tea polyphenol, mixed tea, and one group of 10 hamsters for the negative control. The positive control group was treated three times a week for 15 weeks with 0.5% of the cancer inducing agent 7,12-dimethylbenz[a]anthracene (DMBA). In addition to this treatment, the hamsters in the other groups received 1.5% green tea polyphenol, 0.1% black tea polyphenol, and 0.5% mixed tea as the only source of drinking water for two weeks before the experiment and then throughout the 15 weeks. After the treatments the groups were sacrificed and the pouches were removed. The number of tumors was counted and the length, width, and height of each tumor were measured. The body weight and tea consumption of the subjects was analyzed by a t-test. The tumor numbers were ranked by the Wilcoxon test, and the incidence of lesions was measured by the X² test.

The current research maintains the idea that tea is effective at inhibiting microbes in the mouth. Experiments were performed to determine the anti-bacterial and anti-viral effects of green and white teas. Inquiries have also focused on infusing these teas with oral hygiene products such as toothpaste and gum. The teas used for this research included Celestial, CPNA International, Stash, and Templar. The toothpaste products included Aquafresh, Crest, Colgate, Orajel, Aim, and Tom’s of Maine; while the gum products used were of Orbit and Trident brands. The antibacterial effects of tea extracts along with oral agents was observed through the Kirby-Bauer technique on Streptococcus mutans. The bacteria was plated and allowed to culture. Disks permeated with oral agents, tea, and oral agents with tea were placed onto the plates and allowed to incubate for 24 hours. Zones of inhibition on the Mueller Hinton II agar determined the inhibition of bacteria. A plaque technique was used to determine the anti-viral effects of these products against the T1 virus, which attacks bacteria such as Escherichia coli. For the experiments with oral agents, four tubes were used. A control tube contained 3 ml of water and 1 ml of virus. One of the experimental tubes testing for the effects of tea on virus had 2 ml of water, 1 ml of virus, and 1 ml of tea. Another tube examining the effect of toothpaste alone had 2 ml of water, 1 ml of virus, and 1 ml of toothpaste. A fourth tube testing the effects of tea infused with toothpaste had 1 ml of water, 1 ml virus, 1 ml of tea, and 1 ml of 1% toothpaste solution. All tubes had 4 ml of liquid, followed by addition of 0.3 ml of Escherichia coli. From all the tubes, 0.1 ml of liquid was placed into a tube containing tris buffer and then mixed. A 0.1 ml of this solution was then placed into a tube with overlay agar, mixed and poured over a plate containing underlay agar. Incubation was allowed to take place for 24 hours, after which plaques were visible
on the plates. The plaques, representing the attack of T1 virus on *E. coli*, were counted and compared to the control. Various viral concentrations were observed via suspension of $10^{-1}$, $10^{-2}$, or as a direct amount concentration.

To determine whether individual catechins work better alone, five catechins of green tea were injected separately into a high pressure liquid chromatography (HPLC). Individual catechin peaks were analyzed based on retention time, the time at which a specific compound comes out of the end of the column, and counts per area of each peak. In order to obtain the amount of catechins present in teas, solutions of green and white tea, green and white tea polyphenol, as well as decaffeinated green and white tea, were also injected separately into the HPLC. By comparing the retention times of the individual catechin peaks with the peaks obtained from the solutions, the amount of catechins present in each solution was determined. To detect the anti-viral and antibacterial effects of the catechins, the same experiments were performed using the plaque and Kirby-Bauer methods.

**Results**

**Cytotoxicity.** For normal GN56 and HGF-1 fibroblasts, initial cytotoxicity occurred at 50 µg/ml for both black and green teas. Initial toxicity for carcinoma cells CAL27 and HSC-2 was reported at 25 µg/ml. Initial toxicity with EGCG was much higher for all cell types: normal cells GN56 and HGF-1 had initial levels of 100 µM and 200 µM, respectively; carcinoma cells CAL27 and HSC-2 had initial levels of 25 µM and 50 µM, respectively. Significant values of $p < 0.05$ were reported for all agents. The generation of H$_2$O$_2$ was also determined, as shown in Table 1. More H$_2$O$_2$ was produced by green tea extract.

**Apoptosis.** Cells undergoing apoptosis were visible by changes in morphology and chromatin condensation. In the study by Ahmad, et al., (1997), observations of DNA fragmentation lead to evidence that treatment of A431 cells with green tea polyphenol resulted in apoptosis at all dosage levels. Treatment of individual constituents of EGCG, ECG, and EGC also resulted in apoptosis at the 40 µg/ml dose. The catechin EC did not show any changes. Figure 1 illustrates the morphological changes, which also provided evidence of apoptosis. No changes were visible with the control (Fig. 1, A) or at the 20 µg/ml dose of catechins (Fig. 1, B). At the 40 µg/ml dose, some cells displayed early apoptotic morphology (Fig. 1, C) and at the 80 µg/ml dose, nearly all cells were observed to be in the late apoptotic stage, characterized by chromatin condensation.
Cell Cycle Arrest. Results from the study by Ahmad, et al., (1997) indicated that after 24 hours of treatment with EGCG, the A431 cells were arrested in the G0-G1 phase. An arrest was visible in 51% of cells at the 40 µg/ml dose, and in 74% at the 80 µg/ml dose. As the amount of cells increased in the G0-G1 phase, fewer cells were visible at the S phase. No significant changes were observed in the G2-M phases.

Mixed tea vs. individual constituents. In the study conducted by Li, et al., (1999) all of the positive control hamsters developed oral tumors after the 15 week trial. The negative control group did not develop any tumors. Hamsters that consumed green tea polyphenol, black tea polyphenol, and mixed tea had fewer tumors than the positive control group (p<0.01). Compared to the positive control, the tumor burden was reduced by 79% in the green tea group, 89% in the black tea group, and 95% in the mixed tea group. These results are highlighted in Table 2.

Anti-viral and anti-bacterial studies. Templar white tea proved to be the most effective in experiments performed. The percent loss of the T1 virus was observed after ten minutes of mixing with the white tea extract and after two hours without mixing. Total inactivation was obtained in two hours. Anti-viral effects were also seen in the individual catechins. After determining the amount of each catechin present in the tea solutions (Graph 1), anti-viral effects were also tested in the individual catechins. EGC and EGCG were found to be the primary virus destroyers, inactivating 99% and 56% of the virus, respectively (Graph 2).

The inactivation of the T1 virus was much more substantial when treated with toothpaste, in conjunction with tea extracts. Tom’s of Maine toothpaste proved to be the best at inactivating viruses when added to white tea. More than 99% inactivation was obtained in ten minutes. The infusion of tea into chewing gum yielded over 90% inactivation of viruses. In the individual catechins, anti-viral effects were also observed upon treatment with Tom’s of Maine toothpaste. According to Graph 3, the catechin EGC destroyed almost 100% of the virus after one hour.

Anti-bacterial experiments highlighted more distinct zones of inhibition for toothpastes and gum treated with tea than for the oral agents by themselves. Figure 2.A illustrates that no zones of inhibition were obtained using the toothpastes Aquafresh and Crest alone on Streptococcus mutans. However, when the toothpastes were added to white tea extract, zones were visible. Figure 2.B shows the zones of inhibition attained with Orbit Cinnamint gum by itself and with white tea extract. Bacterial destruction was not observed upon treatment with individual catechins.
Table 1. Generation of H$_2$O$_2$ in cell culture medium amended with test agents

<table>
<thead>
<tr>
<th>Test Agent</th>
<th>Concentration</th>
<th>H$_2$O$_2$ in medium (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGCG</td>
<td>50 µM</td>
<td>20±4.2</td>
</tr>
<tr>
<td></td>
<td>100 µM</td>
<td>45±3.8</td>
</tr>
<tr>
<td></td>
<td>500 µM</td>
<td>100±21.9</td>
</tr>
<tr>
<td>Green tea extract</td>
<td>100 µg/ml</td>
<td>85±12.5</td>
</tr>
<tr>
<td>Black tea extract</td>
<td>100 µg/ml</td>
<td>63±12.2</td>
</tr>
</tbody>
</table>

Table 2. Effects of Tea on Oral Tumor Formation in DMBA-Treated Hamsters$^{a,b}$

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Animals</th>
<th>No. of Tumor Bearing Animals</th>
<th>No. of Tumors per Hamster</th>
<th>Mean Tumor Volume, mm$^3$</th>
<th>Mean Tumor Burden, mm$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Positive Control</td>
<td>16</td>
<td>16</td>
<td>3.81±2.13</td>
<td>98.2±63.0</td>
<td>374.2±239.9</td>
</tr>
<tr>
<td>2) Green tea (1.5%)</td>
<td>16</td>
<td>13</td>
<td>2.18±1.17*</td>
<td>32.3±31.2*</td>
<td>77.0±8.1*</td>
</tr>
<tr>
<td>3) Tea Pigments (0.1%)</td>
<td>16</td>
<td>13</td>
<td>1.88±1.30*</td>
<td>22.8±14.8*</td>
<td>42.9±27.8*</td>
</tr>
<tr>
<td>4) Mixed tea (0.5%)</td>
<td>16</td>
<td>12</td>
<td>1.25±1.12*</td>
<td>13.5±12.1*</td>
<td>16.9±15.1*</td>
</tr>
<tr>
<td>5) Negative Control</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

$a$: Values are means ± SD. DMBA, 7,12-dimethylbenz[a]anthracene.

$b$: Statistical significance is as follows: *, p < 0.01 vs. Group 1 (Wilcoxon test)

Fig. 1. Morphologic changes in A431 cells carcinoma cells grown in control, unamended treatment as evident by confocal microscopy. A) control; B-D) 20, 40, and 80 mg/mL dose of epigallocatechin-3-gallate, respectively, cells grown in control, unamended exposure for 48 hours. Morphologic changes denoting apoptotic cells are shown by arrows. Bar represents 25 µm. Data shown here are from a representative experiment repeated four times with similar results.
Graph 1: Catechin Quantities in Solutions of Green and White Teas

Graph 2: Destruction of T1 Virus by Green Tea Catechins, Green Tea and White Tea Solutions
Graph 3: Effect of Tom's of Maine Toothpaste on Destruction of T1 Virus by Templar Green Tea, Templar White Tea, and Green Tea Catechins

Percent Loss of T1 Virus

<table>
<thead>
<tr>
<th></th>
<th>10 minutes</th>
<th>1 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green Tea</td>
<td>44.1%</td>
<td>99.9%</td>
</tr>
<tr>
<td>Green Tea Polyphenols</td>
<td>99.9%</td>
<td>99.9%</td>
</tr>
<tr>
<td>White Tea</td>
<td>99.9%</td>
<td>100.0%</td>
</tr>
<tr>
<td>White Tea Polyphenols</td>
<td>99.9%</td>
<td>100.0%</td>
</tr>
<tr>
<td>EGC</td>
<td>37.6%</td>
<td>99.9%</td>
</tr>
<tr>
<td>EGCG</td>
<td>9.7%</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

Fig. 2.A

Fig. 2.B
**Discussion**

**Apoptosis and Cell Cycle Arrest.** Scientific studies suggest that consumption of tea from the plant *Camellia sinensis* might prevent oral cancer. In the study conducted by Ahmad, et al., (1997) green tea was shown to protect against cancer by causing cell cycle arrest and inducing apoptosis. Results illustrated that green tea polyphenol as well as individual constituents, including EGCG, ECG, and EGC, induced apoptosis in epidermoid cancer A431 cells. EGCG was discovered to have induced apoptosis in all carcinoma cells, and not in normal cells. Since the carcinoma cells originated from different body sites EGCG may be used to prevent many types of cancers. In addition, if this process of induction can be observed *in vivo*, EGCG may be used as a chemopreventive agent.

Ahmad, et al., (1997) determined that EGCG blocks the G₀-G₁ phase of the cell cycle in epidermoid A431 cells. The arrest suggests that this constituent can be used to control cancer growth. Therefore, by artificially imposing the cell cycle checkpoints, cancer cell growth may be slowed.

**Mixed tea vs. individual constituents.** The study by Li, et al., (1999) showed that tea could inhibit DMBA-induced oral cancer in Syrian hamsters. Tea was shown to inhibit the development of preneoplastic lesions into carcinoma cells. Li, et al., (1999) suggested various mechanisms for this inhibition, including the role of tea in suppressing cell proliferation, thereby protecting cells from DNA damage. Also, the antioxidative and free radical scavenging activities of tea are proposed to protect against oral cancer. The mixed tea was discovered to have the strongest inhibitory effect on tumor development than both green and black tea polyphenol. A mixed solution of tea was also found to have greater anti-viral and anti-bacterial effects than individual tea catechins in the research performed with Dr. Schiffenbauer. These results established a Type II error in my hypothesis, which perceived single constituents of tea to be more active and have more beneficial properties than when mixed.

**Antioxidant vs. Pro-oxidant Effects.** Contrary to popular belief that the catechin EGCG acts as an antioxidant, certain studies suggest that is may also have pro-oxidant activities. In particular, Weisburg, et al., (2004) demonstrated that green tea is even more toxic than black tea. This is most likely a result of higher EGCG levels in green tea. In the study, cultured carcinoma cells infused with EGCG and polyphenolic extracts produced H₂O₂. This generation indicates the pro-oxidant potential of EGCG. Upon 72 hours of exposure to EGCG, carcinoma cells developed cytoplasmic vacuoles, and were found to be more sensitive to oxidative stress than normal cells. In accord with this study, Yang, et al., (1998) discovered that H₂O₂ was generated in H661 lung
cancer cells during incubation with EGCG. This discovery also ascertained a Type II error in my hypothesis, which identified tea constituents as encompassing antioxidant activities. Weisburg, et al., (2004) suggested that the contradictions in findings of EGCG as an antioxidant versus a pro-oxidant may be a result of differences in experimental design, specifically the concentration of EGCG, the cell lines and culture medium used.

**Future objectives.** All studies agree that *in vivo* investigations need to be conducted in order to visualize the effects of tea constituents in humans. Since certain catechins demonstrated anti-viral effects, specifically the catechin EGC, further experimentation can be performed to investigate the role of these catechins when used as additives to agents such pomegranate juice, which is known for its stimulation of the immune system. The constituents can also be infused into oral care strips. These paper strips will dissolve in the mouth, leaving behind the catechin extract. This development may lead to the destruction of bacteria that cause caries and plaque. As additives, these constituents can promote fresh breath, as well as healthier teeth and gums.

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