Chapter I

Prostate Cancer Chemoprevention by Tea Polyphenols: Concepts, Overview and Introduction
Chapter 1

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Concepts, Overview and Introduction

This chapter describes the basic concepts regarding i) prostate cancer (CaP), ii) chemoprevention, and iii) chemopreventive agent of choice; polyphenols obtained from tea plant *Camellia Sinensis*. The detailed account of individual projects including their own introduction, rationale, methods and discussion is provided later with individual chapters.

1.1 Introduction

Cancer is the uncontrolled growth and spread of cells that may affect almost any tissue of the body. More than 10 million people are diagnosed with cancer worldwide every year. It is estimated that there will be 15 million new cases by the year 2020. Cancer causes 6 million deaths every year—or 12% of deaths worldwide. Carcinogenesis is a multistep process driven by genetic and epigenetic alterations that disrupts the regulatory pathways controlling cellular proliferation, programmed cell death (apoptosis), angiogenesis and differentiation (Lippman and Hong, 2002; Vogelstein and Kinzler, 2002; Jones and Baylin, 2002). As a normal routine, mammalian cells grow, divide, and die in an orderly fashion. However, sometimes the normal regulation of growth, division and death of cell is disturbed that leads to a rapid and uncontrolled proliferation of cells ultimately resulting into the development of 'Cancer'.

Cancer has afflicted humans throughout recorded history. It is no surprise that from the dawn of history, a lot has been written about cancer. Some of the earliest evidence of cancer is found among fossilized bone tumors, human mummies in ancient Egypt, and ancient manuscripts. Bone remains of mummies have revealed growths suggestive of the bone cancer, osteosarcoma. In other cases, bony skull destruction as seen in cancer of the head and neck has been found. Oldest description of cancer (although the term cancer was not used) was discovered in
Egypt and dates back to approximately 1600 B.C. The Edwin Smith Papyrus, or writing, describes 8 cases of tumors or ulcers of the breast that were treated by cauterization, with a tool called "the fire drill". This writing stated about the disease, "There is no treatment".

The origin of the word cancer is credited to the Greek physician Hippocrates (460-370 B.C.), considered the "Father of Medicine." Hippocrates used the terms carcinos and carcinoma to describe non-ulcer forming and ulcer-forming tumors. In Greek these words refer to a crab, most likely applied to the disease because the finger-like spreading projections from a cancer called to mind the shape of a crab. Carcinoma is the most common type of cancer.

Specific genetic and epigenetic alterations may affect gene expression and/or the structure and function of specific gene products associated with carcinogenesis. The two major classes of such genes are Oncogene and tumor suppressor genes (TSGs), both of which are essential components of many regulatory circuits. Oncogenes, some of which are the human analogues of viral genes, promote tumorigenesis upon activation by a single event, such as point mutation, chromosomal translocation and/or amplification. Tumor suppressor genes are the genes whose inactivation is associated with tumor development. In contrast to oncogenes, both alleles of a TSG must be affected to promote tumorigenesis (Knudson, 2001). Inactivation of TSG can occur through a combination of genetic and epigenetic mechanisms. Activation of oncogenes and inactivation of TSGs may result in uncontrolled cell growth and acquisition of tumorigenic phenotypes (Hanahan and Weinberg, 2000).

Cell growth, proliferation, apoptosis (programmed cell death), angiogenesis and differentiation are controlled by complex signal transduction pathways (regulatory circuits) (Hanahan and Weinberg, 2000). These pathways are typically activated by the binding of ligand(s) (an extracellular protein or a small molecule, such as steroid hormone) to a specific cell surface or nuclear receptor. Activation and transmission of signal frequently depends on serine or tyrosine kinases associated with the cytoplasmic portion of the receptor. The signal is then relayed into the nucleus by cytoplasmic proteins, resulting in a change in intracellular gene and protein expression patterns. Alteration of critical proteins in these regulatory pathways may have an oncogenic effect. For example, the genes encoding the epidermal growth factor
receptor (EGFR) and the platelet-derived growth factor receptor (PDGFR) are known
Oncogenes that are over expressed or mutated in a number of human malignancies (Yarden, 2001; Blume-Jensen and Hunter, 2001). Similarly, intracellular kinases that
are intermediate effectors in relaying intracellular signals are frequently targets for
oncogenic activation (Frame, 2002; Sawyers, 2002). Inactivation of TSGs involved in
cell signaling may result in malignant transformation as well. For example, the
inactivation of phosphatase and tensin homologue (PTEN), which normally promotes
apoptosis by inhibiting the Akt cell survival signaling pathway, may promote
carcinogenesis in the prostate, breast, ovary, endometrium and brain (Backman et al.,
2002; Mills et al., 2001; Liu et al., 2000).

Factors escalating or influencing cancer risks are varied, acting alone or in
combination, usually over a period of many years. More than half of all cancers are
due to variations in dietary habits, environmental exposures and life style factors
including tobacco use, alcohol consumption, overweight and obesity, lack of physical
activity etc. Besides, genetic predisposition and medical interventions, infectious
agents such as viruses, bacteria and parasites, reproductive and hormonal factors
may also account for increased cancer risk. However, most of the prevalent human
cancers can, to a significant extent, be prevented and many could be avoided by a
suitable choice of lifestyle and environment. Many specific causes of cancer are now
known, the most important being smoking, obesity and a few oncogenic viruses, but a
large proportion of global variation for common cancers such as breast, prostate,
colon and rectum remain unexplained.

1.2 Prostate Cancer

Prostate cancer (CaP), the most common non-dermatological malignancy in
many developing nations as well as in developed countries such as the United States
of America, ranks as the leading cause of cancer death in men (Jemal et al., 2004).
Currently in India, CaP is the fourth most prevalent cancer in men ranking after lung,
stomach and colon. Compared with western countries the incidence of CaP in India
and other Asian countries is less however every 8 men out of 100 thousands will
develop clinical detectible CaP in their life time. According to the projections from
American Cancer Society, in year 2004, 230,110 new CaP cases will be diagnosed in
the US alone and approximately 29,900 CaP-related deaths are predicted in the USA
In 15 years time, CaP is predicted to be the most common cancer in men and thus has become a significant public health concern. The incidence of CaP increases rapidly with advancing age, and multiple genetic and epigenetic factors have been implicated in the oncogenesis of the disease. Despite recent improvements in diagnostic and therapeutic techniques, survival rate of these patients is poor because the majority of the patients are present with advanced stage of the disease at the time of diagnosis.

Death from CaP is usually associated with the development of hormone-refractory metastatic disease (Gittes, 1991; Epstein, 1998). The initiation and progression of CaP involves a complex series of both exogenous and endogenous factors. Although clinical CaP incidence and mortalities vary greatly among populations (Jemal et al., 2004; Setchell et al., 1984), the frequency of latent CaP is evenly distributed. This suggests that external factors are important in the transformation from latent into more aggressive clinical cancers (Adlercreutz et al., 1993; Adlercreutz et al., 1990). Geographic variations and increasing incidence in populations migrating to high incidence areas, demonstrate increasing evidence that lifestyle factors such as diet, physical activity, smoking and other environmental factors may be important in the etiology of invasive CaP (Jemal et al., 2004, Carlstrom and Stege, 1997; Nomura and Kolonel, 1991).

Most cancers have a latency period of 10 to 20 years, which provides ample time for preventive measures (Kelloff et al., 1999). In most epithelial tissues, including the prostate, genetic progression and loss of cellular control functions occur as the cell and tissue phenotype changes from normal to dysplasia (prostatic intraepithelial neoplasia [PIN]), to increasingly severe dysplasia high-grade PIN (HGPIN), then to superficial cancers, and finally to invasive disease. These changes occur over a long time period as CaP is known to have a long latency period (Kelloff et al., 1999; Boone et al., 1992). Specifically in the prostate, PIN develops over approximately 20 years. Progression from PIN to HGPIN and early latent cancer may take 10 or more years, and clinically significant carcinoma may not occur for another three to 15 years (Kelloff et al., 1999; Boone et al., 1992). The features of prostate cancer including high prevalence, long latency, significant mortality and morbidity, provide the most promising opportunities for chemoprevention. The reason is that CaP is a disease with high latency period and is typically diagnosed in men over the age of 50. The present life expectancy in the world is around 78 years and those who live up to an
age of 65 will have an average of 13 more years thus increasing the incidence of the disease.

1.3 Cancer Chemoprevention

Centuries old proverb in English, “An ounce of prevention is worth a pound of cure”, though not said for Chemoprevention, is probably the basis behind this emerging concept in cancer management.

Dr. Michael Sporn coined the term Chemoprevention (pronounced as KEE-mo-pre-VEN-shun) in the 1970s, as part of his pioneering effort to encourage research into preventing cancer before it begins rather than treating tumors once they appear. Chemoprevention, by definition, is a means of cancer control in which the occurrence of the disease can be entirely prevented, slowed or reversed by the administration of one or more naturally occurring and/or synthetic compounds (Wattenberg, 1990; Ames, 1983; Morse and Stoner, 1993; Sporn, 1991; Ames and Gold, 1990; Kohlmeier et al., 1997). The expanded definition of cancer chemoprevention also includes the chemotherapy of precancerous lesions which are called preinvasive neoplasia, dysplasia or intraepithelial neoplasia, depending on the organ system (Boone et al., 1990; Boone et al., 1992). Because of this expanded definition of chemoprevention some degree of overlap between chemotherapy and chemoprevention also occur. For example, when an established cancer is cured by chemotherapy, chemoprevention could prevent its recurrence and any further development of new cancers. Thus, in recent years the concept of chemoprevention has matured to an extent that it appears real to achieve significant reversal and/or complete to partial suppression of pre-malignancy to malignancy at several sites by chemopreventive agents.

Ideally chemopreventive compounds should have a) little or no toxic effects, b) high efficacy in multiple sites, c) capability of oral consumption, d) a known mechanism of action, e) low cost, and f) human acceptance. Chemoprevention of cancer thus differs from cancer treatment in that the goal of this approach is to lower the rate of cancer incidence. This approach is promising because the therapy and surgery have not been fully effective against the high incidence or low survival rate of most of the cancer types. Furthermore, this approach appears to have practical implications in reducing cancer risk because unlike the carcinogenic environmental factors that are difficult to control, individuals can make decisions to modify their
choice for the food and beverage they consume. In recent years, the naturally occurring compounds, especially the antioxidants, present in the common diet and beverages consumed by human population have gained considerable attention as chemopreventive agents for potential human benefit (Wattenberg, 1990; Ames, 1983; Morse and Stoner, 1993; Sporn, 1991; Ames and Gold, 1990; Kohlmeier et al., 1997). Abundant epidemiological, experimental, and metabolic studies have provided convincing evidence that nutrition plays an important causative role in the initiation, promotion and progression stages of several types of human cancers (Wattenberg, 1990; Ames, 1983, Boone et al., 1992). It has become clear that, in addition to substances that pose a cancer risk, the human diet also contains agents which are capable of affording protection against some forms of cancer (Ames, 1983; Boone et al., 1990; Surh, 2003; Kohlmeier et al., 1997). This collective information strongly suggest that the occurrence of cancer can be prevented or slowed by dietary intake of substances that have the capacity to afford protection against the occurrence of cancer.

Some of the chemopreventive agents that demonstrate potential for cancer are drugs, biologics, and micronutrients. For all cancer targets, successful chemopreventive strategies require well-characterized agents, suitable cohorts, and reliable intermediate biomarkers of cancer for evaluating chemopreventive efficacy. Agent requirements include experimental or epidemiological data that show chemopreventive efficacy, safety on chronic administration, and a mechanistic rationale for the observed chemopreventive activity. According to conventional classification originally proposed by Lee Watenberg, chemopreventive agents are subdivided into two main categories- blocking agents and suppressive agents (Wattenberg, 1985). Blocking agents prevent carcinogens from reaching specific target sites, from undergoing metabolic activation or from subsequently interacting with crucial cellular macromolecules (DNA, RNA and Proteins). Suppressing agents, on the other hand, inhibit the malignant transformation of initiated cells in either the promotion or the progression stage. Chemopreventive phytochemicals have an ability to block or reverse the premalignant stage (initiation and promotion) of multi stage carcinogenesis.

In recent years, attention has been focused on the role of nutrients as chemopreventive agents. The concept of using micronutrients for the chemoprevention of cancer is based on the evidence from human epidemiology, the results of a few clinical trials, and studies of animal carcinogenesis models for cancer-inhibiting potential of these
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substances. Basic research has identified nutrients as agents that inhibit mutagenesis and hyperproliferation, as well as those that induce apoptosis or differentiation as critical characteristics for chemoprevention regardless of their specific molecular targets. Some of the most promising nutrients identified as chemopreventive agents in prostate cancer include phytoestrogens/isoﬂavones, vitamins D and E, dietary phytochemicals, selenium and lycopene.

The research aimed towards the prevention of cancer development began in mid 1950s corresponding to the time when the researchers were busy understanding the process of carcinogenesis. However, the ﬁeld of chemoprevention became more prominent and focused in late 1960’s, when Lee W. Wattenberg, one of the founders of the ﬁeld, conceptualized this strategy and suggested a mechanistic framework to the diverse array of agents identiﬁed (Wattenberg, 1966; 1985 and 1992). He initially called the concept of cancer chemoprevention as ‘chemoprophylaxis of carcinogenesis’ (Wattenberg, 1966).

The chemoprevention research efforts of National Cancer Institute of the USA, regarded as one of the best cancer research organizations, started in the early 1980s and have grown considerably since that time. Currently, approximately 400 compounds are being studied as potential chemopreventive agents, mainly in laboratory research. Over 40 of these compounds are being studied in clinical trials. However the biggest challenge remains to ﬁnd the answers for: a) which chemopreventive agent(s), b) what form and route of administration, and c) which population. Once we are able to ﬁnd the satisfactory answers to these questions, the reduction of cancer through chemoprevention could become a reality.

In recent years, naturally occurring compounds, especially the antioxidants, present in the common diet and beverages consumed by human population have gained considerable attention as chemopreventive agents for potential human beneﬁt (Wattenberg, 1990; Ames, 1983; Morse and Stoner, 1993; Sporn, 1991; Ames and Gold, 1990; Kohlmeier et al., 1997). Abundant epidemiological, experimental, and metabolic studies have provided convincing evidence that nutrition plays an important causative role in the initiation, promotion and progression stages of several types of human cancers (Wattenberg, 1990; Ames, 1983; Boone et al., 1992). It has become clear that, in addition to substances that pose a cancer risk, the human diet also contains agents which are capable of affording protection against some forms of cancer (Ames, 1983;
Ames and Gold, 1990; Kohlmeier et al., 1997). This collective information strongly suggest that the occurrence of cancer can be prevented or slowed by dietary intake of substances that have the capacity to afford protection against the occurrence of cancer.

1.3.1 Prostate cancer chemoprevention

Evidences from geographic, epidemiological and in vitro and in vivo experiments suggest that environmental carcinogenic factors and nutrition play important causative role in the initiation, promotion and progression stages of CaP (Hebert et al., 1998; Rose, 1996; Hayward et al., 1998). Thus, chemoprevention appears to be a useful strategy for the management of CaP. This is well-supported by the epidemiological observation that the Japanese and Chinese populations, which are habitual drinker of several cups of tea, have one of the lowest rate of CaP in the World (Wynder et al., 1994; Kohlmeier et al., 1997).

CaP is believed to be an ideal candidate disease for chemoprevention because of its high latency period and as it is typically diagnosed in men over the age of 50. Thus, even a slight delay in the progression of this disease by chemoprevention could result in a substantial reduction on the incidence of the disease and more importantly, improve the quality of life of the patients with the disease (Klein & Thompson, 2004; Wynder et al., 1994). The identification of promising agents (and their molecular targets) for CaP prevention is guided by data derived from a variety of sources. These evidence-based leads come from (a) epidemiological observations, (b) prostate cancer treatment trials, (c) secondary analyses from large, randomized, controlled cancer prevention trials, (d) an understanding of cancer biology and prostate carcinogenesis, and (e) experimental animal models.

1.4 Chemopreventive Agents

Observations based on statistical and epidemiological data suggest that dietary factors stimulate the development, growth and spread of tumors, and transform normal cells into malignant ones. These are regarded as suspected carcinogens, however there is also accumulating evidence from population as well as laboratory studies to support an inverse relation between regular consumption of fruit and vegetables and the risk of specific cancers. Certain foods, including many vegetables,
fruits and grains, offer protection against various cancers. Chemoprevention researchers try to find substances--either in food or pharmaceuticals--that can prevent or halt carcinogenesis. Although it is said that there is no 'magic bullet' that can completely conquer cancer but using chemopreventive agents many types of the disease could be avoided. It has been estimated that more than two-thirds of human cancer could be prevented through appropriate lifestyle and dietary modifications (Surh, 2003).

1.4.1 Dietary phytochemicals as chemopreventive agents

'Phyto' is from the Greek word meaning plants and thus phytochemicals are non-nutritive components in the plant-based diet that possess substantial anticarcinogenic and antimutagenic properties. It is believed that people who eat five servings of fruits and vegetables have half the risk of developing cancer than those who have three or less.

The NCI has identified about 35 plant-based foods that possess cancer-preventive properties. These include tea, turmeric, garlic, soybeans, ginger, onion, tomato and various cruciferous vegetables including cabbage, broccoli, cauliflower and Brussels sprouts (Figure 1). Ongoing and performed studies have shown the validity of use of these phytochemicals in cancer chemoprevention (Surh, 2003). Evidence suggests that a diet high in fruits and vegetables may decrease the risk of chronic diseases, such as cardiovascular disease and cancer, and phytochemicals including phenolics, flavonoids and carotenoids from fruits and vegetables may play a key role in reducing chronic disease risk (Surh, 2003; Ames, 1983). In the laboratory, phytochemicals have been found to have very strong antioxidant activity, inhibit cancer cell proliferation, decrease lipid oxidation, and lower cholesterol. Phytochemicals, include quercetin, catechin, phloridzin and chlorogenic acid, all of which are strong antioxidants.

Many population-based studies have highlighted the ability of macronutrients (for example, carbohydrates, fat, proteins, fibres etc.) and micronutrients (vitamins and trace metals etc.) that are present in the vegetables and fruits to reduce the incidence of cancer. However, plants contain numerous chemical substances other than these micronutrients that might also be useful in preventing cancer.
Figure 1: Representative chemopreventive phytochemicals and their dietary sources.
These micro- and macro- nutrients involve numerous cellular molecules and events in their act of chemoprevention. The cellular and molecular events affected or regulated include carcinogen activation/detoxification by xenobiotic metabolizing enzymes, DNA repair, cell-cycle progression, cellular proliferation, differentiation and apoptosis, expression and functional activation of oncogenes, angiogenesis and metastasis, and hormonal and growth-factor activity (Surh, 2003; Mukhtar & Ahmad, 1999). Despite these remarkable advances, the identification of molecular and cellular targets of chemopreventive phytochemicals is still incomplete. Many of the molecular alterations that are associated with carcinogenesis occur in cell-signaling pathways that regulate cell proliferation and differentiation.

1.5 The beverage Tea: an overview

Tea, made from the leaves of Camellia sinensis, an evergreen shrub of the Theaceae family, is a beverage of choice in many countries around the world. Consumption of tea can be traced back to 2737 B.C., according to a Chinese legend, it was Shen Nung a legendary Emperor known as the Divine Healer, who discovered the refreshing effects and fine taste of tea when he was out camping and a few Camellia leaves chanced to fall into boiling water. The Chinese started cultivating this plant and it subsequently spread to Japan, Java, India, Sri Lanka etc. as tea became an increasingly popular beverage. Currently tea plant is being cultivated in approximately 30 countries. According to an estimate of the Food and Agriculture Organization of the United Nations, world tea production in 2002 reached 3 million tons and the demand for tea has been growing ever since. For many thousand years, the harvesting and processing of the leaves of the tea plant Camellia sinensis, has become as an integral part of human society and traditional culture. Because of its characteristic flavor and pharmacological properties, next to water, tea is the most popular beverage consumed worldwide (Harbowy and Balentine, 1997). The per capita consumption of tea in the United States of America is approximately 340 g. Although the largest total consumption of tea is registered in India (540,000 metric tons, 620 g per capita), Ireland has the largest per capita consumption of tea (3220 g).
1.5.1 Consumption, Composition and Chemistry of Tea

1.5.1.1 Consumption

Tea plant originated in Southeast Asia and is presently cultivated in over 30 countries around the globe and currently tea beverage is consumed worldwide, although at greatly varying levels. Consumption of tea is far from uniform. A large segment of the world's population virtually consumes no tea. Not only does the tea consumption vary from country to country, but also there is enormous variation in any given population. This ranges from none to as many as 20 or more cups per day. Although firm data is not available, it is generally accepted that next to water, tea is the most consumed beverage in the world; with a per capita worldwide consumption of approximately 120 ml per day (Katiyar and Mukhtar, 1996). Approximately 2.5 million metric tons of dried tea is annually manufactured in the whole world of which, 78% is black tea, 20% is green tea and less than 2% is oolong tea. Green tea is produced in relatively few countries, and is mainly consumed in China, Japan, India, and a few countries in North Africa and Middle East. About 78% of the world's total tea consumed is black tea, which is mainly consumed in the western countries and some Asian countries. Oolong tea production and consumption is confined to southeastern China, and Taiwan (Katiyar and Mukhtar, 1996).

1.5.1.2 Composition

The composition of tea-leaf varies with climate, season, horticultural practices, variety of the plant, and age of the leaf, i.e. the position of the leaf on the harvested shoot. Three main varieties of the commercial tea are available: Green (unfermented), Oolong (partially fermented) and Black (fully fermented). Their composition varies according to the manufacturing process which differs in the degree of 'enzymatic oxidation' or fermentation. In Table 1 most important polyphenolic components present in a typical green and black tea beverage is shown but variations may be considerable (Katiyar and Mukhtar, 1996). Oolong tea composition in general falls in between green and black teas.

Green tea. The manufacturing process of green tea involves rapid steaming or pan frying of freshly harvested leaves to inactivate enzymes, prevent fermentation and thereby producing a dry stable product. Green teas are generally produced in two
Table 1. Major polyphenolic constituents present in tea leaves (%wt/wt).

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>GREEN TEA</th>
<th>BLACK TEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechins</td>
<td>30-42</td>
<td>3-10</td>
</tr>
<tr>
<td>Flavonols</td>
<td>5-10</td>
<td>6-8</td>
</tr>
<tr>
<td>Other flavanoids</td>
<td>2-4</td>
<td>-</td>
</tr>
<tr>
<td>Theogallin</td>
<td>2-3</td>
<td>-</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Quinic acid</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>Theanine</td>
<td>4-6</td>
<td>-</td>
</tr>
<tr>
<td>Methylxanthines</td>
<td>7-9</td>
<td>8-11</td>
</tr>
<tr>
<td>Theaflavins</td>
<td>-</td>
<td>3-6</td>
</tr>
<tr>
<td>Thearubigens</td>
<td>-</td>
<td>12-18</td>
</tr>
</tbody>
</table>
different varieties: White tea and Yellow tea, the latter is less fermented because of the increasing popularity of green tea, a wide variety of green tea products are coming in the market. Epicatechins are the main constituent compounds in green tea, giving it the characteristic color and flavor.

**Black tea and Oolong tea.** When producing black and oolong tea, the fresh leaves are allowed to wither until the moisture content of leaves is reduced to about 55% of the original leaf-weight that results in the concentration of polyphenols in the leaves and the deterioration of leaf-structural integrity. This step gives the typical aroma to the tea. The withered leaves are rolled and crushed, initiating fermentation of the polyphenols. This process is known as maceration and the fermenting mass is known as 'dhool'. The process used to macerate the leaf plays an important role in the final grading of tea. During these processes the catechins are converted to theaflavins and thearubigins. Theaflavins are astringent compounds contributing importantly to the color and taste of the black tea. The thearubigen fraction is a mixture of substances, with a molecular weight distribution of 1,000-40,000 and account for 15% of dry weight solids of black tea.

Oolong teas are prepared by firing the leaves shortly after rolling to terminate the oxidation and dry the leaves. Normal oolong tea is considered to be about half fermented compared to black tea. Oolong tea extracts contain catechins at a level of 8-20% of the total dry matter. The fermentation process results in the oxidation of simple polyphenols to more complex condensed polyphenols to give black and oolong teas their characteristic colors and flavors (Harbowy and Balentine, 1997).

### 1.5.1.3 Tea Chemistry

The chemical composition of green tea is approximately similar to that of the fresh leaf with regard to the major components. Green tea contains polyphenolic compounds, which include flavanols, flavandiols, flavonoids, and phenolic acids. These compounds account up to 30% of the dry weight of green tea leaves. Most of the polyphenols present in green tea are flavanols, commonly known as catechins. Some major catechins present in green tea are (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG). The
chemical structures of these compounds are given in Figure 2. In addition, caffeine, theobromine, theophylline, and phenolic acids such as gallic acids are also present in green tea (Table-1).

During the fermentation process involved in the manufacture of black tea, the monomeric flavan-3-ols undergo polyphenol oxidase-dependent oxidative polymerization leading to the formation of bisflavanols, theaflavins, thearubigins, and some other oligomers. Theaflavins (1-2%, on dry weight basis) contains benzotropolone rings with dihydroxy or trihydroxy substitution systems. About 10-20% of the dry weight of black tea is due to thearubigens, which are even more extensively oxidized and polymerized. The structures of theaflavins and thearubigins are shown in Figure 2.

Oolong tea contains monomeric catechins, theaflavins, and thearubigins. In addition, epigallocatechin esters, theasinensins, dimeric catechins, and dimeric proanthocyanidins are also the characteristic components of oolong tea.

1.5.2 Health beneficial effects of tea

For many thousand years, the harvesting and processing of the leaves of *Camellia sinensis*, popularly known as tea in the whole world, has become as an integral part of the human society and culture. Next to water, tea is the most popular beverage consumed worldwide preferably because of its characteristic flavor and pharmacological properties (Harbowy and Balentine, 1997). Although the largest total consumption of tea is registered in India (540,000 metric tons, 620 g per capita), Ireland has the largest per capita consumption of tea (3220 g). The per capita consumption of tea in the USA is approximately 340 g. Tea contains several polyphenolic components, which are antioxidant in nature, and many studies have shown that tea polyphenols possess the ability to prevent oxidant-induced cellular damage (Harbowy and Balentine, 1997; Katiyar and Mukhtar, 1996). In recent year studies, from many laboratories worldwide, conducted in various organ specific animal bioassay systems have shown that tea and the polyphenolic constituents isolated from it, are capable of affording protection against a variety of cancer types. Although majority of the studies is conducted with green tea, a limited number of studies have also shown the anti-cancer efficacy of black tea.
Figure 2: Chemical structures of major Green and Black tea polyphenols. R= galloyl group.
Tea is consumed worldwide for different reasons ranging from improving blood flow, combating cancer and cardiovascular disease, eliminating various toxins, and improving resistance to various diseases. Supportive scientific evidences for these claims, surfacing in recent times have led to an increase in the consumption of green tea. Much emphasis is being placed on events at the cellular level due to its strong antioxidant activity. Several studies have suggested that the polyphenols, present in green tea possess high antioxidant activities, which in turn, protects cells against the adverse effects of damaging reactive oxygen species (ROS) that are constantly produced in the body. ROS, such as superoxide radical, hydroxyl radical, singlet oxygen, hydrogen peroxide, peroxynitrite, and alkoxyradicals, damage lipids, protein, and nucleic acids, and cellular components such as ion channels, membranes, and chromatin, this in turn leads to cellular injury and cellular dysfunctions. These ROS are known to contribute to the etiology of many chronic health problems including cardiovascular diseases, inflammatory diseases, diabetes, obesity and cancer (Katiyar and Mukhtar, 1996; Liao et al., 2001). Wei et al. (1996) have shown that the polyphenolic constituents of tea can act as scavengers of ROS and thereby prevent damage to cellular macromolecules. The scavenging activity of the specific catechin molecules is related to the number of o-dihydroxy and o-hydroxyketo groups, solubility, concentration, the accessibility of the active group to the oxidant, and the stability of the reaction product (Liao et al., 2001).

Some of the effects of tea polyphenols may also be due to the chelation of metal ions. Tea manifests chelating activity in vivo as indicated by the fact that tea consumption lowers absorption of dietary iron in controlled feeding studies and decreases body iron balance (Liao et al., 2001). Also, this chelating activity is important since it protects iron-loaded hepatocytes from lipid peroxidation by removing iron from these cells. The study has shown that EGCG may chelate the cations, which may contribute to its ability to inhibit angiotensin-converting enzyme. Polyphenols of tea chelate copper ions and this mechanism has also been suggested to protect low-density lipoproteins from peroxidation. Because of its chelating properties tea may additionally protect against toxicity due to heavy metals (Liao et al., 2001). Catechins may also affect signal transduction pathways, modulate many endocrine systems, and alter hormones and other physiological
processes as a result of their binding these metals/enzyme co-factors (Kao et al., 2000). The beneficial effects of tea consumption in health and the associated mechanisms by which it provides health benefits are summarized in table 2.

### Table-2: Beneficial effects of tea consumption

<table>
<thead>
<tr>
<th>Disease</th>
<th>Evidence in Humans/Animals</th>
<th>Possible Targets/Mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td>Strong/ Moderate</td>
<td>Ability to inhibit the oxidation of LDL. Decrease the plasma phosphatidylcholine hydroperoxide level. Improve brachial artery dilation. Lower hypercholesterolemia to normal levels and reduce blood pressure.</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Suggestive/Moderate</td>
<td>Inhibit the formation of sugars. Repress glucose production and phosphoenolpyruvate carboxykinase and glucose-6-phosphatase gene expression. Antihyperglycemic effects. Protect against cytokine- mediated damage of pancreatic β-cells.</td>
</tr>
<tr>
<td>Obesity</td>
<td>Suggestive/Moderate</td>
<td>Decline in food intake. Lower the blood levels of glucose and insulin. Increase the 24-hour energy expenditure. Reduce high-fat diet-induced body weight gain.</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>Some/None</td>
<td>Increase bone mineral density.</td>
</tr>
<tr>
<td>Arthritis</td>
<td>Suggestive/Some</td>
<td>Reduction of inflammatory mediators, neutral endopeptidase activity and IgG levels.</td>
</tr>
<tr>
<td>Neurological</td>
<td>Some/None</td>
<td>Inhibit tyrosinase. Inhibit COMT and Prolylendopeptidase. APP secretion and protection against Abeta toxicity.</td>
</tr>
<tr>
<td>Bacterial</td>
<td>Suggestive/Some</td>
<td>Modulation in composition of microflora. Inhibit the growth of clostridia and promote bifidobacteria colonization. Decrease ration of oxidants. Inhibiting the</td>
</tr>
</tbody>
</table>
1.5.3 Tea and cancer chemoprevention

Cancer is believed not to be a single disease but rather a conglomeration of several diseases. Most of the work on chemoprevention of cancer by tea has been conducted using green tea or its individual polyphenolic constituents, especially EGCG, which is a major constituent of green tea. Green tea catechins act as antioxidants and inhibit the growth of cancer in experimental animal models. This raises the possibility that consumption of green tea or its catechins may lower cancer risk in humans. Several epidemiological studies conducted so far have verified this suggestion. EGCG inhibits the action of enzymes to prevent the activation of procarcinogens, resulting in their inactivation and finally excretion (Lin et al., 1999). As shown by McArdle et al. (1999) consumption of both green tea and black tea aqueous extracts influences the excretion of mutagens and promutagens in the urine of animals. In animal studies, the polyphenolic fraction isolated from green tea, the water extract of green tea, or individual polyphenolic antioxidants present in green tea have been shown to afford protection against chemically induced carcinogenesis in lung, liver, esophagus, pancreas, forestomach, duodenum, colon, and breast (Ahmad et al., 1996, Katiyar and Mukhtar, 1996, Liao et al., 2001). From some recent studies conducted, it is now believed that much of the cancer chemopreventive properties of green tea are mediated by EGCG, but other polyphenolic constituents may also possess similar effects (Ahmad et al., 1996; Katiyar and Mukhtar, 1996; Mukhtar and Ahmad, 1999). However, it is yet to be decided whether the different polyphenolic constituents of green tea work through similar or different mechanisms.

Outcome of several epidemiological studies have suggested that tea and its associated compounds prevent certain types of cancers (Liao et al., 2001). This is understandable as cancer is a complex disease with multiple etiologies, even for one body site. Therefore, it seems to be a false hope that any single nutritional or synthetic agent can prevent or treat all types of cancer. However, based on a large volume of cell culture, animal studies, and human observational studies, there is a hope that tea consumption can retard cancer development at selected sites in some populations. The challenge is to find these populations that could reap the benefit.
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Green tea users have an approximate 50% reduction in risk for both esophageal cancer (Gao et al., 1994) and stomach cancer (Yu et al., 1995). Inhabitants of tea-producing districts in Japan have a lower mortality due to stomach cancer, possibly due to regular consumption of green tea (Oguni et al., 1989). In addition to regular drinking of tea, the Japanese population consumes green tea in all types of products, including candy, gums, bread, and many other edible products. Green tea was found to be linked to a reduced risk of oral cancer in northern Italians and a Chinese population; esophageal cancer in Chinese women; gastric cancer in Swedish adolescents; pancreatic cancer in residents of a retirement community in the USA; and colon cancer amongst retired male self-defense officials in Japan (Kono et al., 1991; Liao et al., 2001; Schwarz et al., 1994). Cohort studies suggest a protective effect of green tea for colon, urinary bladder, stomach, pancreatic, and esophageal cancer (Bushman, 1998). In a Japanese population survey, an overall protection together with a slowdown of the increase of cancer incidence with age was reported (Imai et al., 1997). The effects were found to be more pronounced when the consumption of tea was over 10 cups per day. According to a study, consumption of seven or more cups per day of green tea significantly decreased the risk of stomach cancer (by 31%) compared with no green tea consumption (Inoue et al., 1998). Regular drinkers of tea experienced a 12% and 53% lower incidence of cancer among males and females, respectively, compared with non-tea drinkers (Ji et al., 1997). When the intake of tea exceeded 200 g/month (dry weight of tea prior to brewing), the risk reduction remained unchanged among women, whereas the incidence of pancreatic cancer was further decreased by 43% in men (Ji et al., 1997). Another case-control study from Poland reported a significant reduction in risk of pancreatic cancer with increasing lifetime consumption of tea (Zatonski et al., 1993). An increased consumption of green tea was closely associated with a decreased number of axillary lymph node metastases among premenopausal patients with stage I and II breast cancer and overall decreased recurrence of stage I and II breast cancer (17% for individuals drinking more than five cups and 24% for those drinking less than four cups.

Recent studies have shown that green tea polyphenols inhibit the growth and progression of prostate cancer in transgenic adenocarcinoma of mouse.
Prostate (TRAMP) model that mimics human disease (Gupta et al., 2001). Prostate cancer is an ideal cancer type for prevention by green tea because the disease is typically diagnosed in older men and thus even a modest delay in disease development could produce a substantial benefit (Gupta et al., 2001).

References


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