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Tea (*Camellia sinensis* (L.)): A Putative Anticancer Agent in Bladder Carcinoma?

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Abstract: The leaves of *Camellia sinensis* (L.) are the source of tea, the second most consumed beverage worldwide. Tea contains several chemical compounds such as polyphenols (mainly catechins), caffeine, theophylline, L-theanine, among many others. Polyphenolic compounds are mainly responsible for its significant antioxidant properties and anticarcinogenic potential. Bladder cancer is one of the most common types of cancer, and its progression and onset are thought to be controlled by dietary and lifestyle factors. Epidemiological studies showed that the regular consumption of tea can be a preventive factor for this type of cancer, and several *in vivo* and *in vitro* studies reported that tea and its components may interfere in the cancer cells' signaling, preventing the bladder tumor progression. The mechanisms responsible for this protection include deregulation of cell cycle, induction of apoptosis while protecting the surrounding healthy bladder cells, inhibition of metastization processes, among others. Herein, we discuss the potential beneficial effects of tea and tea components in bladder cancer prevention and/or treatment, and how they can be helpful in finding new therapeutic strategies to treat this type of cancer.

Keywords: Bladder cancer, caffeine, *Camellia sinensis*, catechins, EGCG, polyphenols.

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INTRODUCTION

Camellia sinensis (L.), commonly known as the tea plant, has been used for many centuries in traditional medicine. The infusion prepared by using its leaves or buds is known as tea. The main types of tea obtained from this plant are green tea (GT), white tea (WT), black tea (BT) and oolong tea (OT). This classification is based on differences in the manufacture and preparation processes, which result in distinctive chemical compositions. Recently, tea's ability to stimulate the immune system and mitigate several diseases has raised great interest [1-10], and these beneficial properties have been attributed to its chemical composition.

Cancer constitutes one of the greatest challenges in terms of developing preventive, therapeutic and diagnosis methods [11]. Phytomedicine is now considered a helpful research area for battling this burden [12-14]. In this context, chemopreventive and chemotherapeutic properties of tea have been studied, and new data arise to unveil the molecular mechanisms by which tea and its components exert their actions. Bladder cancer is among the most common types of cancer and it can appear under different forms, being the transitional cell carcinoma (TCC) the most frequent [15, 16]. The majority of TCC tumors are superficial papillary tumors, and only 10 to 15% of the cases evolve to a much more aggressive non-papillary phenotype, that invades the muscle wall of the bladder (for review see [15]). In 2008, nearly 400.000 new cases and 150.000 deaths were estimated due to this type of cancer, that most frequently affects men [17].

Many factors are pointed out as risk factors for the development of this disease (for review see [15]). These include smoking [16, 18, 19], exposure to diesel and combustion fumes [18], genetic components account the widely accepted health benefits of tea, studying its [16, 18, 20] and liquid ingestion [20, 21]. Of note, it has been reported that the consumption of milk [21], BT, GT [20] and some alcoholic beverages [20, 21], decreases the risk of developing bladder cancer. This type of cancer is thought to be preventable by modification of dietary factors [22]. Therefore, and having in effects on bladder cancer can yield new insights on the treatment and prevention of this disease. Herein, we present an up-to-date overview of the potential beneficial effects of tea and its components in the prevention and treatment of bladder cancer.

CLASSIFICATION OF TEAS

Tea is one of the most popular beverages worldwide and is produced by infusion of the leaves or buds of the tea plant (*Camellia sinensis*). This beverage has been used in eastern traditional medicine for many centuries, since it yields several health benefits [4, 9, 23]. *C. sinensis* can originate four major types of tea, depending on the tea leaves' harvesting and processing [4, 9, 23]. Upon harvesting, the leaves suffer an enzymatic oxidation process, also called "fermentation" [4, 9]. The enzyme involved in this process, polyphenol oxidase (PO), is mainly responsible for the differences in the phenolic profiles of the several types of tea. Its action can be inactivated by quickly heating the leaves or buds, a post-harvesting technique commonly used in the production of GT and WT [4, 9]. GT, BT and OT are all obtained from *C. sinensis* mature dried leaves, but they possess different chemical compositions. Consequently, some very obvious organoleptic differences, namely in taste, color and flavor are also noted. In the production of GT [24], the mature leaves are harvested and then quickly heated, to inactivate PO and prevent oxidation. On the other hand, the production of BT [25], includes crushing the leaves, which are then allowed to "ferment" for two hours, and heated afterwards. Production of OT is similar to the latter; however, the leaves are only allowed to "ferment" for one hour before being heated [4, 9]. WT, the most expensive and rare type of tea, is produced from the tips or leaf buds not fully opened, which are quickly heated to prevent withering and oxidation [4, 9]. Thus, WT's chemical composition is similar to that of *C. sinensis* buds and young leaves.

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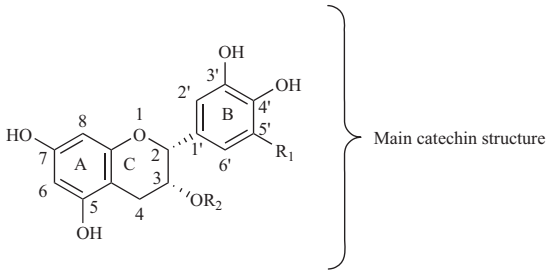


	R1	R2
(-)-epicatechin	H	H
(-)-epigallocatechin	OH	H
(-)-epicatechin-3-gallate	H	Gallate group
(-)-epigallocatechin-3-gallate	OH	Gallate group

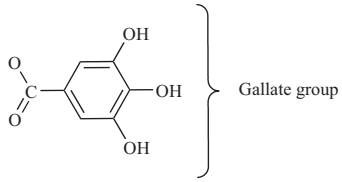


Fig. (1). Chemical structures of the main tea catechins. These compounds are mainly constituted by two aromatic rings (A, B) and a dihydropyran heterocyclic ring (C). The flavan-3-ol epicatechin is constituted by an ortho-di-hydroxyl group in the B ring (at carbons 3' and 4') and a hydroxyl group in the C ring (at carbon 3), and its ester derivative epicatechin-3-gallate possesses an additional gallate moiety esterified in the C ring, at carbon 3. Epigallocatechin contains three hydroxyl groups in the B ring (at carbons 3', 4' and 5') and its ester derivative epigallocatechin-3-gallate additionally possesses an esterified gallate at the carbon 3 of the C ring.

CHEMICAL COMPOSITION OF TEAS

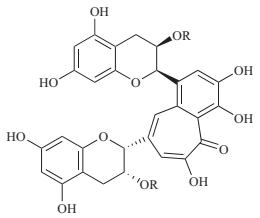
Tea's chemical composition is very complex, containing polyphenols, proteins, polysaccharides, amino acids, minerals, trace elements, methylxanthines and organic acids, among others [4, 9]. The chemical composition of tea may be affected by several factors such as geographical origin, climate, growing conditions, harvesting practices, maturity stage of the plant and manufacturing processes [4, 9, 26].

Tea Catechins

Polyphenols are the most abundant and active group of compounds present in tea, and are thought to be the responsible for much of the health benefits attributed to this beverage [6, 27, 28]. Flavonoids are amongst the major classes of phenolic compounds, from which is important to highlight the flavan-3-ol family. The members of this family are also known as catechins. There are various catechins in tea, such as (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG) and (-)-epigallocatechin-3-gallate (EGCG) [9, 23]. These compounds are thought to possess a very high antioxidant power [28-30].

The health benefits attributed to catechins are mainly due to their chemical structure. Catechins are essentially comprised of three rings (the aromatic rings, A and B, linked to a dihydropyran heterocyclic ring, C) and are characterized by multiple hydroxyl groups on the A and B rings [31] (Fig. **1**). Their chemical differences are due to the presence of different groups attached to those rings [4, 9, 31]. In EC, we can find an ortho-di-hydroxyl group in the B ring and a hydroxyl group in the C; ECG, contains a gallate moiety esterified in the C ring. EGC possesses a trihydroxyl group on the B ring, and EGCG possesses an esterified gallate on the C ring [4, 31]. GT and WT present higher catechin content, while OT and BT possess in high quantities other phenolic compounds [4, 25, 26, 32]. The enzyme PO, released during the crushing of the leaves for production of BT and OT, catalyzes the oxidation and polymerization of the catechins, producing theaflavins and thearubigins [25, 26].

Theaflavins (Fig. **2**) are comprised of the bicyclic benzotropolone ring, and result of the catechins' dimerization. In turn, thearubigins possess oligopolymeric structures and are thought to be the result of the hydroxylation of theaflavins, but are still poorly chemically characterized.



	R
Theaflavin	H
Theaflavin-3,3'-digallate	Gallate group

Fig. (2). Chemical structures of the main theaflavins. Theaflavins result from the dimerization of catechins and are constituted by a skeleton comprised of the bicyclic benzotropolone ring. The majority of theaflavins are formed from an epicatechin and an epigallocatechin. Theaflavin-3,3'-digallate is produced by dimerization of epicatechin-3-gallate and epigallocatechin-3-gallate.

The chemical structure of tea's components is particularly associated with its antioxidant properties. Studies suggested a relationship between the content of pyrogallol and hydroxyl groups and the superoxide anion scavenging ability, as well as between the presence of galloyl moieties and the ability to quench hydroxyl radicals [9, 33]. Also, the number and position of the hydroxyl groups on the molecules influences the antioxidant ability of flavonoids [31]. Tea catechins such as EGCG lack a 2, 3 double bond and a carbonyl group at the 4-position, a combination that strengthens the antioxidant activity [9]. However, the higher total phenolic component may not always be correlated with greater antioxidant capacity, mainly because different phenolic profiles can yield different responses [34]. OT and BT have a lower catechin concentration but they are also important sources of health promoting substances and have considerable antioxidant properties. An analysis of commercial tea extracts showed that BT and OT may have great importance in processes such as hydroxyl radical scavenging and nitric oxide suppressing [26].

Among the most important contributions of tea to health are its antioxidant [4, 7, 23, 35], anti-inflammatory, antimicrobial, antimutagenic, antimetastatic and anticarcinogenic activities [4, 8, 10, 23, 35-39]. Moreover, studies suggested that regular tea intake is potentially helpful in protecting against many chronic diseases, such as cancer, and in enhancing the immune function [1, 6, 23, 27]. Among the tea catechins, EGCG is considered the most abundant and active [4, 9, 23, 27, 40], and its beneficial effects have been widely studied. Its anticancer potential has been reported as one of its most important properties [35, 38, 41-47]. Nevertheless, aside from the polyphenolic content, there are other tea components that are thought to contribute to the improvement of health associated with tea consumption. Caffeine, L-ascorbic acid, L-theanine, quercetin, among others, may be crucial for the beneficial effects attributed to tea consumption [4, 9, 48].

Tea Methylxanthines

Caffeine (Fig. **3**) is the main methylxanthine present in tea (ranging between 1.0% and 3.5% in tea preparations [26, 40]). Other methylxanthines present in tea are theophylline and theobromine.

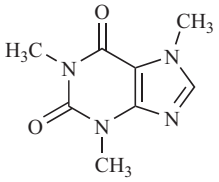


Fig. (3). Chemical structure of caffeine (1,3,7-trimethylpurine-2,6-dione). It is a naturally occurring tea purine derivative with three methyl groups at positions 1, 3 and 7.

Of note, caffeine is one of the most consumed substances in the world [49]. Tea is thought to be the second major source of caffeine in the diet of North American adult population [50]. Due to its chemical stability, caffeine levels in tea are not altered by "fermentation" [25]. Still, the levels of caffeine present in each tea type remains a matter of debate [26, 32, 51]. The discrepancies may be due to different extraction conditions, distinct analytical methods and the variability of the plants.

There are many adverse effects associated with excessive caffeine consumption (for review, see [50]). Studies reported death provoked by excessive intake of caffeine, although it is rare [50]. It was proposed that reducing the caffeine content in GT may help to highlight its beneficial properties for human health [52]. Similarly to tea catechins, caffeine also has different effects at cellular and metabolic levels (for review, see [53]). Its most important mechanism of action involves selectively blocking the adenosine receptors and competitively inhibiting the action of adenosine in the cells, resulting in an increased release of hormones such as norepinephrine, dopamine and serotonin [50].

Caffeine's anticarcinogenic properties were also documented. A study reported apoptosis induction after the topical application of caffeine in skin tumors in rats, with virtually no effects on normal tissue cells. The mechanisms were most likely p53 independent, since the tumor cells displayed p53 mutations [54]. Others suggested that this compound promotes cell cycle arrest in the G0/G1 phase in cancer cells, most likely through suppression of cyclin D1-cdk4 complex activation and consequent inhibition of retinoblastoma protein (pRb) phosphorylation, in a dose-dependent manner, suppressing the tumor proliferation without inducing cell apoptosis [49]. Particularly in studies of tea consumption by humans, the importance of caffeine in tea preparations was highlighted, since decaffeinated teas presented very low (or even inactive) cancer inhibitory properties [5]. Besides, the intake of decaffeinated coffee may be related with an increased risk for developing bladder cancer [20]. However, data on the role of caffeine on tea-associated health benefits are scarce and much work needs to be done.

TEA AND BLADDER CANCER

The manifestation of cancer is due to a large set of contributing factors which makes it very difficult to identify the exact causes, biomarkers or treatments [16]. Although more studies are required, especially regarding human bladder cancer, tea and its individual components remain a field of interest in many studies [2, 3, 6, 35, 36, 39, 42, 46-48, 55-58]. Also, several studies reported that tea can act against bladder cancer in different situations, as a preventive or therapeutic factor. After GT ingestion by mice, tea catechins (namely EGCG) can be widely distributed through the body but they concentrate in specific organs, particularly in the bladder [36]. However, the anticarcinogenic properties of tea in human bladder cancer, although predictable, still lack strong supporting evidence. It was reported, by a number of studies, the beneficial role of tea as a protective or preventive factor against this disease in humans [20, 59, 60].

The selected studies performed on this matter (Table **1**) include case-controls [18, 20, 21, 59, 61-65] and cohort studies [19, 22, 60, 66] and are mostly based on the statistical analysis of questionnaires filled by patients or former patients, regarding their dietary and lifestyle habits in the years anteceding the cancer onset. Some studies suggested that regular tea consumption can be either a risk factor for bladder cancer [64] or a preventive factor [20, 59, 60]. Other studies reported that tea consumption has no association with the disease triggering, development or outcome [18, 19, 21, 22, 61-63, 65, 66]. Although these studies are very important, there are also some drawbacks to be considered. The use of questionnaires makes the studies highly dependent on the subjects' interpretation or past memory raising doubt about the veracity, due to the subjects' forgetting or deliberately tampering the facts. Besides, most of these studies also include a complicated analysis of data, ranging from type and duration of the beverages consumed, fruit and vegetable consumption, smoking status, among others. Of note, some of the studies do not refer the type of tea investigated [18, 59-61, 65], which hinders any association between consumption of one tea type and bladder cancer development. Finally, most of the analysis were performed in very different populations, which greatly vary in terms of age, countries and habits, making very difficult the extrapolation of results and conclusions.

Regarding animal model and *in vitro* studies, the conclusions are more elucidating, although there is still work to be done. GT showed some promising results in preventing and treating several types of cancer, including bladder tumors, and its identification as a key antimutagenic factor dates as far as 1985 [37]. Mainly acting

Table 1. Epidemiological studies regarding regular tea consumption and human bladder cancer. The types of tea, studies and results obtained by the authors are resumed.

		Type of Tea Consumed	Main Conclusions			Main Outcomes
			Risk factor	Protective or preventive action	No association	
Epidemiological studies	Population-based cohort	**GT** [19]			✖ [19]	na [19]
	Case-control	**GT** [20, 21, 64] **BT** [20, 21, 62-64] **OT** [64] **Unspecified** [18, 65]	✖ [64]	✖ [20]	✖ [18, 21, 62, 63, 65]	↑ In patients that consumed GT or BT daily (1 cup or more), for a period over 30 years. [64] ↓ In patients with daily consumption of GT and BT (1 cup or more). [20] na [18, 21, 62, 63, 65]
	Cohort	**GT** [22] **BT** [22, 66] **Unspecified** [60]		✖ [60]	✖ [22, 66]	↓ In postmenopausal women who consumed more than 2 tea cups daily. [60] na [22, 66]
	Population-based case-control	**Unspecified** [59, 61]		✖ [59]	✖ [61]	↓ In subjects with low fluid intake who consumed more than 5 tea cups per day. [59] na [61]

Legend: BT – Black tea; GT – Green tea; OT – Oolong tea; ↓ - Reduced number of cases of bladder cancer; ↑ - Increased number of cases of bladder cancer; na – No statistically significant association. Superscript numbers are references as indicated in references section.

through its polyphenol components, particularly catechins, it was demonstrated the GT ability to prevent the initiation and growth of bladder tumors in rats [43, 58, 67], inhibit bladder tumor development and invasion *in vitro* (in some cases showing positive synergistic effects with other substances) [43, 48] and protect normal bladder cells, while killing the malignant ones [43, 46]. Although catechins alone are a powerful tool to oppose cancer development, GT extract and dried leaves are also very helpful and a practical way to treat cancer. They possess numerous components with anticancer benefits, being less expensive, widely available and safe [67]. However, there is some controversy about its exact mechanisms of action and effects. Several authors suggested that GT does not induce morphological changes in cultured bladder cancer cells, acting only through modification of matrix components for growth inhibition [48], while others reported, in the same circumstances, morphological alterations accompanied by apoptosis in cancerous cells [43].

Despite BT's lower concentration of catechins, a study in rats with N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN)-induced bladder cancer that consumed BT instead of water for 40 weeks reported a significant decrease of tumor volume, although GT showed even more positive results [67]. Regarding studies focused in consumption by humans, BT is one of the most studied types of tea (see Table **1**). A case-control study in 40 human bladder cancer patients from southern Taiwan and 160 subjects that did not displayed bladder cancer or any other urological disease, suggested that the regular drinking of GT or BT for a period over 30 years may increase the risk of bladder cancer [64]. In contrast, a population-based case-control study in 1452 bladder cancer patients and 2434 control subjects from Iowa, reported a preventive/protective role in the subjects that drank more than 5 five cups of "tea" per day. Though the tea type was not specified, the authors highlighted that it may be mostly BT, once it is thought to be the most consumed tea in western countries [59]. It was also reported the probable protective role of daily consumption of one or more cups of BT and GT in a case-control study of 1007 human patients with confirmed bladder cancer, without treatment previous to the study, and 1299 healthy control subjects [20]. However, most studies focused on tea consumption found no significant association between consumption of BT and bladder cancer in humans [21, 22, 62, 63, 66].

Regarding OT, there is not much information on bladder cancer related studies. A case-control study of southern Taiwan patients reported no significant association between daily OT consumption and bladder cancer [64]. On the other hand, a study focused on consumption of OT by rats with induced bladder cancer showed that regular OT intake, instead of water, decreased the bladder tumor volume [67].

Concerning WT, as far as we know, there are no available studies on its effect on human bladder cancer patients. WT possesses nearly the same components as GT, although in different amounts. Thus, this type of tea is expected to present bladder cancer related health benefits. Moreover, the action of WT against other types of cancer is well documented [8], as well its superior antioxidant properties comparing to other types of tea [1, 4, 7, 8]. Overall, although there is some controversy, most of the studies suggested that tea and its phytochemical components may be a good strategy to prevent and treat bladder cancer. Nevertheless, more studies are needed to clarify the molecular mechanisms by which tea and its phytocomponents act.

MOLECULAR MECHANISMS IN CHEMOPREVENTION AND THERAPY OF BLADDER CANCER

C. sinensis can be used to yield teas with remarkable health promoting benefits. The chemopreventive and chemotherapeutic potentials of this beverage are well documented, but tea can exert

its anticancer effects through different mechanisms (for review see [68]). Tea catechins and other components possess anticarcinogenic properties that have been tested *in vitro*. However, the metabolism of catechins may alter their function and/or bioavailability, hampering its beneficial activities [69]. Tea components were also proved to be promising chemopreventive agents *in vivo*. Nevertheless, most of those mechanisms are not yet fully understood, and the identification of the exact components that contribute to those effects is far from being disclosed. Controversy aside, it is widely accepted that tea components, particularly EGCG and other catechins, are crucial for its chemopreventive activity. Some pathways and targets of tea and its components, to exert their anticarcinogenic activities, were identified (Fig. **4**). These studies were mainly focused on their antioxidant activity or specific cell signaling pathways.

Antioxidant and Pro-Oxidant Activities

Oxidative stress is a major contributor to cancer development and progression [70]. It occurs when there is an imbalance between the production of ROS and reactive nitrogen species (RNS) and the levels or activity of antioxidant defenses. Failure to maintain this equilibrium can lead to the destruction of important biomolecules and ultimately to cellular damage. Despite its critical significance in signaling mechanisms, reactive species can induce DNA lesions, which in turn may produce mutations, known to be on the basis of cancer onset [16, 70]. Besides, ROS/RNS can react with cellular proteins and lipids, yielding products with carcinogenic and mutagenic properties [70-73]. These events can promote significant alterations to cells' signaling, regulation and gene expression [71, 73].

It was showed that the urine of patients with either bladder or prostate cancer exhibited high levels of the oxidative stress marker 8-hydroxydeoxyguanosine. This molecule is produced when ROS cause the hydroxylation of DNA's guanine and is excreted in urine upon DNA repair, therefore being known as an indicator of DNA repair and cellular oxidative stress [74]. Thus, that study illustrates that the DNA damage induced by oxidative stress may be an important pathological feature of bladder cancer patients.

The structure of tea catechins has a major influence in their antioxidant properties [28, 31, 75]. However, these compounds are relatively unstable, and it is common for catechins to suffer oxidation processes. This oxidation can either be performed by catechins themselves (known as auto oxidation), or can be catalyzed by transition metals such as copper and iron [75]. Studies reported that antioxidant properties of tea catechins were only observed *in vivo* when animals were under oxidative stress, contrary to *in vitro* studies, where these activities could nearly always be observed. Catechins were also suggested to influence the levels of endogenous antioxidants. A study performed on Wistar rats showed that continuous administration of EGCG (2 mg per kg of body weight) for 30 days was able to significantly improve animals' antioxidant defenses, ameliorating the oxidative stress levels in their brains, induced by age [76]. EGCG successfully induced a rising in the activity levels of the following antioxidant enzymes: superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase, as well as in the levels of nonenzymatic antioxidants such as L-ascorbic acid, α -tocopherol and glutathione. Also, lipid peroxidation and levels of protein carbonyls (markers of protein damage induced by ROS) showed significant decrease after EGCG treatment. Moreover, treatment with EGCG, in young Wistar rats whose brain tissue did not displayed oxidative stress levels induced by age, did not yield the same significant alterations in the antioxidant levels [76].

The beneficial properties of tea catechins can be extended to many different types of cancer, since the carcinogenic events and properties of tumor cells are fairly similar in many of them. For example, catechins and other tea polyphenols were reported to

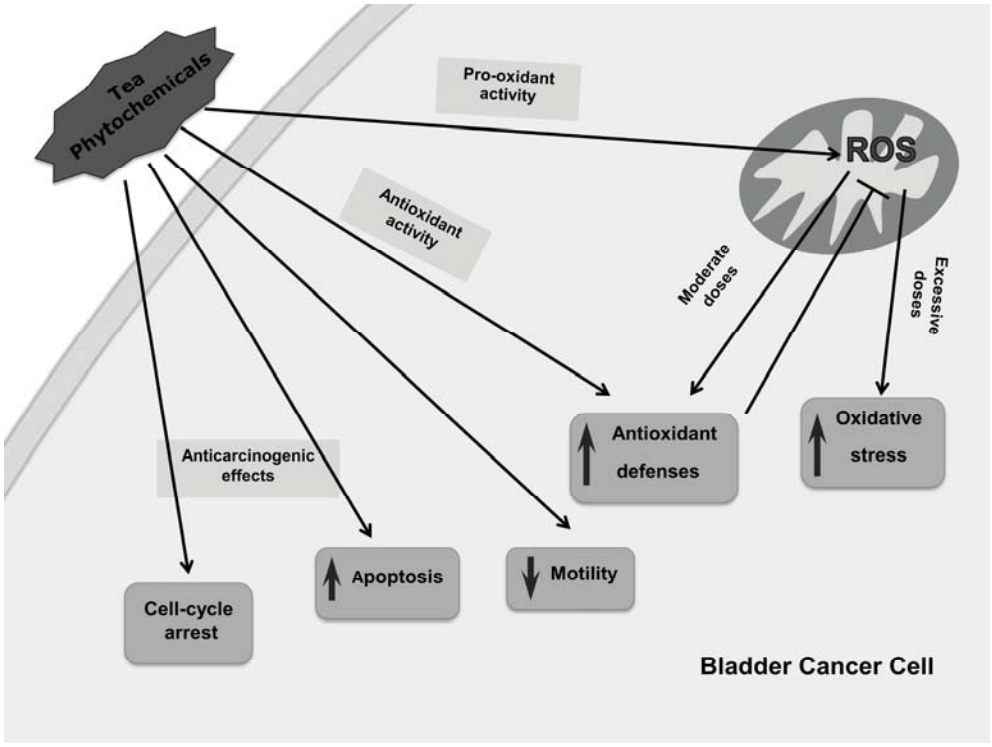


Fig. (4). Schematic illustration of the main effects of tea components in a bladder cancer cell. Tea's phytocomponents can exert antioxidant or pro-oxidant activities, depending on its concentrations. When in high doses, they can induce excessive reactive oxygen species (ROS) production by mitochondria, contributing to oxidative stress increase. On the other hand, when in moderate doses, they contribute to low production of ROS, which will provoke a response in the cell, augmenting the endogenous antioxidant defenses. These compounds can also interfere in multiple cell signaling pathways, promoting cell cycle arrest, inducing cell apoptosis and decreasing cells ability to migrate.

protect against skin [10, 44, 54], renal [2], hepatic [3] and lung [8, 42] cancers. Still, in the context of bladder cancer, knowledge and proof of all these activities by tea catechins are still lacking. *In vitro* studies in normal/cancerous bladder cell lines exposed to hydrogen peroxide (H_2O_2), an oxidative agent, analyzed the antioxidant effects of the treatment with GT extract (14% polyphenols), polyphenon-60 (PP-60, 60% pure catechins), ECG and EGCG and concluded that ECG, EGCG and PP-60 were able to improve cell survival. The protection afforded against apoptosis induced by H_2O_2 was higher for normal bladder cells than in cancerous ones. H_2O_2 exerted its apoptotic effects in normal bladder cells mainly by inducing ROS generation. These effects were either partially mediated by the superoxide anion or by direct H_2O_2 signaling. Although the exact mechanisms by which PP-60 and the selected catechins were able to diminish the damaging effects of H_2O_2 were not thoroughly explained, it was hypothesized that these compounds can modulate cellular gene expression, possibly by causing the induction of protein kinase C and downregulation of nuclear factor kappa beta. These alterations in cell signaling would suppress cell death mechanisms and counterbalance ROS production [46].

Of note, tea catechins also possess the ability to generate ROS. The mechanisms of hydrogen atoms/electron transfer and oxidation processes catalyzed by transition metals often originate highly reactive species, such as quinone, semi-quinone radicals and other free radicals (for review see [75]). For instance, the incubation of several carcinoma cell lines with EGCG resulted in inhibition of phosphorylation and reduced protein levels of epidermal growth factor receptor (EGFR) and HER-2/neu, both members of the ERBB receptor family [77]. These effects were delayed with addition of the enzyme superoxide dismutase, suggesting that they may be, at least partially, a result of the action of EGCG oxidation products [77]. Therefore, these compounds can exhibit either antioxidant or pro-oxidant properties, which are based on complex chemical interactions (for review see [31, 75]). This fact is an important feature to consider not only when fighting a pathological state, but also due to toxicity that is observed when high doses of tea polyphenols are administered. For instance, moderate doses of polyphenols induce low ROS production and activate the nuclear factor Nrf2, which can then translocate to the cell nucleus and stimulate the expression of antioxidant enzymes [78]. On the other hand, excessive amounts of polyphenols will produce higher levels of ROS. Treatment of CF-1 mice with a single oral dose of 1500 mg/kg EGCG reduced the animals' survival by 85% and the administration of daily doses of 500 and 750 mg/kg decreased survival by 20% and 75%, respectively. High doses of orally administered EGCG may induce hepatocyte toxicity and even mortality in mice, in a dose and time-dependent manner. These events were suggested to be caused by EGCG induction of oxidative stress [79].

Tea catechins may have nefarious effects on normal cells, or even activate certain tumor survival pathways. For example, the catechins ECG and EGCG were capable of activating hypoxia-inducible factor 1 in a human breast cancer cell line, responsible for aiding tumor development and survival under hypoxic conditions. This was due to their ability to chelate iron ions, given that activation was blocked when this metal was added [80]. More recently, a study reported that EGCG, when used to treat human lung and skin cell lines, was responsible for an increase in DNA damage, genetic mutation frequency and apoptosis. These effects were attributed to EGCG's reductive ability. It was suggested that this catechin may have more detrimental action in healthy cells than those of oxidative species, such as H_2O_2, and known highly toxic chemotherapeutic agents, such as cisplatin [81].

Cell Signaling Alterations and Other Reported Mechanisms

The numerous and complex signaling pathways that exist in a cell are extremely important to maintain its homeodynamics and normal functioning. The disclosure of these pathways has become very important in the study of several diseases. Cancer cells normally display several differences in metabolism [82], gene expression and survival mechanisms, among others. Thus, as expected, tea and its components can inhibit carcinogenesis through a wide variety of mechanisms (for review see [68, 83]). These compounds may induce apoptosis, deregulate cell cycle, inhibit growth, proliferation, angiogenesis, enzyme activities and gene transcription, among many other mechanisms (for review see [83]). These events are controlled by a series of different pathways that may interact amongst themselves, making this subject very complex and requiring a thorough analysis, which is not yet completely disclosed [83].

Concerning bladder cancer, some studies were performed *in vivo* and *in vitro* (Table **2**), elucidating possible mechanisms by which tea and its individual components exert their chemopreventive effects [83, 84]. *In vitro* studies were performed in many different bladder cancer cell lines, and reported the anticarcinogenic effects of tea components (mainly EGCG). The effects of treatment with increasing concentrations of EGCG in rat bladder TCC cells and in mouse leukemia cells are well studied. DNA ladder assays confirmed that cell survival decreased in an EGCG time and dose-dependent manner in both cell lines. Moreover, histological observations revealed cellular shrinkage, pyknosis and cell surface blebbing, also verified in another study [43, 45]. Contrary to leukemia cells, the bladder cancer cell line showed weak banding pattern on the DNA ladder assay. Still, cell survival decrease was confirmed, illustrating that the anticarcinogenic effects of EGCG in these cells may not include apoptosis induction as a primary mechanism of action [45]. Analysis of DNA fragmentation was also performed on NBT-II bladder tumor cells treated with different concentrations of EGCG. The cells displayed growth inhibition and evidence of cell cycle arrest in the G0/G1 phase. Further analysis revealed that EGCG treatment induced a significant downregulation of *CCND1* gene expression (which encodes the cyclin D1 protein), decreased expression of cyclin D1, cdk4 and cdk6 proteins and also decreased hyperphosphorylation of pRb, illustrating that EGCG may interfere in cyclin D1-cdk4/6-Rb protein machinery, causing cells' cycle arrest [55]. Similar results were reported using other bladder cancer cell lines, which showed a significant growth inhibition and decrease in cell migration/invasion assays, induced by EGCG [38, 43]. EGCG activated the p42/44 MAP kinase pathway and consequently its signaling target STAT3, which is implicated in several models of malignant tumor progression. However, in other studies, inhibition of these pathways did not alter the reduction in cellular migration induced by EGCG, suggesting that these may not be the sole mechanisms through which EGCG exhibits its antimetastatic effects [38]. Further studies revealed that EGCG inactivated the Akt kinase pathway, also implicated in cellular migration, and that this mechanism may be underlying the observed antimetastatic activity of EGCG [38, 43]. Indeed, antimetastatic properties of EGCG could also be observed in other studies, and may be related with interference in cellular adhesion mechanisms since it was demonstrated a reduced expression of N-cadherin, β -, and ɣ -catenin proteins in cells treated with EGCG [38]. Besides Akt inactivation, others also correlated the apoptotic effects of EGCG treatment on bladder cancer cells with the reported decrease in heat-shock protein 27 and Bcl-2 protein (involved in the inhibition of cell apoptosis), and the increase in Bad and Bax levels, known proapoptotic proteins [43]. These events may also be responsible for increased activity of caspases 3 and 9 in bladder cancer cells treated with EGCG, further illustrating that EGCG activates the apoptotic mitochondrial pathway [43].

The suggestion that EGCG is capable of inducing cell apoptosis was also supported by other studies, performed in murine bladder cancer cells treated with EGCG combined with gold nanoparticles

that showed that this combined treatment successfully reduced tumor cell viability, increased the number of apoptotic bodies formed, decreased the levels of antiapoptotic Bcl-XL, increased the levels of proapoptotic proteins such Bad and Bax and increased the expression levels of caspases 3 and 7. Altogether, these findings also suggest an involvement of mitochondrial apoptotic pathway in the EGCG mechanism of action [56].

As discussed, the majority of *in vitro* studies report different effects of tea (and particularly EGCG), ranging from induction of apoptosis, cell cycle arrest and inhibition of cell proliferation, among others. Also, alterations in many different proteins and mechanisms were verified after cancer cells treatment. Despite interfering in seemingly different and unrelated pathways, certain effects of EGCG may explain many of its different anticancer activities. In molecular modeling studies, the main intracellular targets of tea and its components reported so far include membrane tyrosine kinase receptors such as HER-2/neu and EGFR, which EGCG can inactivate by direct binding to the active tyrosine kinase sites, modifying protein conformation or altering the lipid rafts [77, 85-87]. Avoiding receptor activation modifies cell signaling pathways and consequently alter gene transcription and protein activity. Interference in those receptors may inactivate the downstream signaling proteins such as Akt and ERK1/2 [87], thus modulating their related pathways. This results in the altered expression of p53 and p27 proteins, along with other known cell cycle regulators and may cause cell cycle arrest [87]. Another result of the interference in the Akt activation is the dephosphorylation of forkhead transcription factors FOXO and the proapoptotic Bad protein, among other downstream signaling proteins [88]. This may cause suppression of angiogenesis [89], enhance the expression of proapoptotic proteins and inactivate antiapoptotic proteins such as Bcl-XL, thus activating cell apoptosis [87]. Therefore, we hypothesize that interference in Akt activation is a tremendously important property of EGCG and may be the base of its anticancer abilities, reflected in many different cell mechanisms, due to the several pathways in which Akt participates.

Nowadays researchers are already able to chemically synthesize catechin analogs, through series of complex chemical reactions [90, 91], in order to stabilize their structure and enhance their properties. Studies reported that protecting the hydroxyl groups in the rings using acetate groups stabilizes the catechins structure and yields effective prodrugs [92, 93]. Also, chemical enhancement of radical scavenging ability of catechin analogs has been achieved by altering the bonds between the B and C rings and the overall geometry of the catechins structure [94]. However, to our knowledge, such compounds have not yet been tested in bladder cancer cell lines. Nevertheless, their anticancer properties were reported in several cancer types. For example, treatment of leukemia, breast and prostate cancer cells with EGCG analogs showed that these compounds were more effective at inducing apoptosis in cells than natural EGCG [93]. Concurrently, others treated melanoma, breast, lung and colorectal cancer cells with natural purified EGCG and ECG analogs [95]. Similarly to previous studies, synthetic catechins displayed higher apoptotic and antiproliferative properties. Also, smaller quantities of the analogs were needed to achieve these results, compared to ones used for EGCG treatments [95].

Importantly, although polyphenols are the major chemical components of tea, its beneficial effects may also be exerted by other constituents. Although only caffeine is present in tea, both caffeine and pentoxifylline showed positive synergistic effects on treatment of bladder cancer cells with the alkylator drug Thiotepa, which is commonly used for treating bladder cancer [96]. After treatment with these methylxanthines, survival of the cells previously treated with Thiotepa decreased significantly. Further analysis revealed that these methylxanthines may prevent G2 cell cycle delay, which is a normal defense mechanism that allows the cells to repair their DNA after Thiotepa aggression. In this way, lethal chromosomal aberrations increased and cell death was provoked [96].

GT extract, alone [97] and combined with a mixture of lysine, proline, arginine, L-ascorbic acid and N-acetyl cysteine [48] also showed positive effects *in vitro*. Treatment of bladder tumor cells with different concentrations of GT extract (comprised of 43.0% EGCG and 13.7% ECG, among smaller quantities of other catechins) resulted in induction of cellular actin polymerization, a protein that forms the cells' microfilaments and is typically depolymerized in cancer cells, thus enhancing cell adhesion and inhibiting motility. This mechanism may be due to a GT induced increase in Rho activity, a regulator of actin stress fiber formation [97]. In another study, GT extract (containing 80% polyphenols, 30% catechins and 1% caffeine, among other components) also showed antimetastatic effects. Analysis of cell proliferation, protein expression and invasive potential in bladder cancer cells treated with different concentrations of the mixture revealed significant inhibition of cell invasion and a dose-dependent decrease in secretion of metalloproteinases 2 and 9, which are enzymes typically secreted by highly metastatic cancer cells that allow them to destroy components of the extracellular matrix and migrate to other locations in the tissues [48].

In vivo studies of tea effects on bladder tumors were performed on mice [38, 43, 56, 57] and rats [45, 58, 67] (see Table **2**). Fortunately, some of the effects of tea components observed *in vitro* have been, to some extent, also reported *in vivo*. EGCG was added to drinking water (0.05% w/v) and consumed by 6 week old BALB/c nude mice, 7 days before subcutaneous injection of bladder cancer cells and 15 days after the referred injection. Results showed significant decrease in tumor volume. Also, no side effects were observed, aside from a slight weight gain in the treated mice [38]. In another study using female C3H/He mice with BBN-induced bladder cancer, which were fed with a solution of GT polyphenols (in a concentration of 0.5%), histological and immuno-histochemical analysis of urinary bladder tissues revealed that a 24 week treatment with GT polyphenols reduced tumor growth and microvessel density. These results illustrate that these compounds also possess antiangiogenic effects, which may be responsible for the reduction in tumor growth, although no specific mechanistic studies were performed [57].

A more recent study also demonstrated inhibition of tumor growth in BALB/c nude mice fed with EGCG (in concentrations of 25 and 50 mg/kg per day) during a 42 days period after cancer induction. The mechanism of action proposed includes activation of intrinsic mitochondrial apoptotic pathway and is based on *in vitro* studies, discussed above [43]. Significant reduction of tumor growth was also reported in bladder tumor-induced male mice treated with EGCG conjugated with gold nanoparticles. This result was accompanied by a decrease in cellular vascular endothelial growth factor expression, a protein known for stimulating vasculo-genesis and angiogenesis. These findings suggest that, besides the apoptotic effects demonstrated by the conjugated treatment *in vitro*, the combination of EGCG plus nanoparticles may also be responsible for an angiogenesis inhibition *in vivo*, most likely through suppression of vascular endothelial growth factor. Nevertheless, the exact mechanisms are still unclear [56]. In a study using male Wistar rats with BBN-induced bladder cancer, animals treated with GT extract and powdered GT leaves displayed significant decrease in tumor volume. Moreover, histological observations revealed an improvement of histological grade of the induced bladder tumors and tendency to decreased depth of invasion [67]. Ingestion of GT powdered leaves (2.5% mixed into a feeding pellet) before and after cancer induction in male Wistar rats resulted in a significant decrease in the number of tumors per rat and in mean volume per tumor, relatively to

Table 2. Summary of the main effects observed in several *in vivo* and *in vitro* studies focused on the effects of tea and its phytochemicals in bladder cancer.

		Tea/ Compound Tested	Effects Observed							
			Tumor size	Metastization	Angiogenesis	Apoptosis	Cell cycle arrest	Morphological changes	Cell cytotoxicity	Chromosome damage
In vivo Studies	**Mouse Model**	**EGCG** [38, 43, 56]	↓	↓	↓	↑	nd	nd	nd	nd
		GT polyphenols [57]	↓	↓	nd	nd	nd	nd	nd	nd
	Rat Model	**EGCG** [45]	↓	nd	nd	nd	nd	nd	nd	nd
		Powdered GT leaves [58, 67]	↓	↓	nd	nd	nd	nd	nd	nd
		GT [67]	↓	↓	nd	nd	nd	nd	nd	nd
		OT [67]	↓	↓	nd	nd	nd	nd	nd	nd
		BT [67]	↓	nd	nd	nd	nd	nd	nd	nd
In vitro Studies (bladder cancer cell lines)	**NBT-II** [55], **J82** [38], **UM-UC-3** [38], **EJ** [38], **KK47** [38], **T24** [38], **TCCSUP** [38], **TSGH-8301** [43], **AY-27** [45], **L1210** [45]	**EGCG**	↓	↓	nd	↑	↑	↑	nd	nd
	T24, TCCSUP, SW780 TCC, RT4, Urotsa [46]	**PP-60+EGCG +ECG (after insult with H_2O_2)**	nd	nd	nd	↓	nd	nd	nd	nd
	T24 [48, 96]	**Lysine+proline +arginine+ ascorbic acid+ GT extract** [48]	nd	↓	nd	nd	nd	nd	nd	nd
		Caffeine/ pentoxifylline+ Thiotepa [90]	nd	nd	nd	nd	↑	nd	↑	↑
	HUC-PC, MC-T11 [97]	**GT extract**	nd	↓	nd	nd	nd	nd	nd	nd

Legend: BT - black tea; ECG – epicatechin-3-gallate; EGCG - epigallocatechin 3-gallate; GT - green tea; OT - oolong tea; PP-60 - polyphenon-60; ↓ - Reduced/inhibited; ↑ - Increased; nd - not determined. Superscript numbers are references as indicated in references section.

controls, as well as improvement of histological grade of the tumors [58]. Treatment of Fisher 344 rats with a solution of 200 μM EGCG also induced a significant decrease in tumor growth [45]. The authors suggested that, while EGCG itself is effective as antitumor agent, its isolation and administration to patients would be difficult, mainly due to economic issues. Hence, the administration of GT leaves seems to be a better solution to counteract bladder cancer, providing an excellent quantity of other beneficial compounds such as other catechins, caffeine, quercetin and vitamins [58, 67].

Concerning *in vivo* and *in vitro* research studies, many promising results have been obtained (see Table **2**). Indeed, tea extracts and components, either natural or synthetic, showed great anticancer activities. For example, studies performed on cancer cell lines demonstrated the efficacy of GT extracts, catechins extracted from tea and synthetic catechin analogs. However, these studies present some cons that may hamper the extrapolation of the conclusions to human health applications, such as the fact that the concentrations of catechins (and other bioactive compounds) tested *in vitro* are normally much higher than those verified *in vivo*. Moreover, differences between animal species subjected to research and humans may also hamper the correct interpretation, extrapolation and practical application of the results and conclusions.

CONCLUDING REMARKS

Tea is one the most consumed beverages in the world. Its beneficial health effects, for so long advertised by traditional medicine, are now being more deeply investigated by modern science. Several studies attributed to tea the ability to protect against infections, chronic diseases and cancer. Tea catechins, the main phenolic compounds and the most active, are thought to be responsible by tea's antioxidant and chemopreventive abilities, which include induction of apoptosis in cancer cells, inhibition of metastization and angiogenesis, cell cycle arrest and enhancement of antioxidant defenses. Bladder cancer is thought to be preventable by modification of dietary factors, and tea consumption has been proposed as a possible defense against this cancer type. However, the fact that tea consumption is indeed an asset in bladder cancer chemopreventive and chemotherapeutic fields still raises some controversy. The molecular mechanisms by which tea and/or its phytochemicals exert the possible protective effects remain unclear. A key feature of cancer cells is proliferation, which has been associated with changes in cellular metabolism. Although some studies showed the potential effect of tea and its components to change cancer cells metabolism, it remains to be tested in bladder cancer cells. Moreover, cell cycle alterations induced by tea consumption and tea phytochemicals in bladder cancer cells should also be explored. Nevertheless, tea's health benefits seem undeniable, and its ability to inhibit tumor spreading and metastasis was verified in *in vitro* and *in vivo* carcinogenic models. The complex mechanisms by which tea and its components may exert their benefits are still poorly understood, and a general consensus has not been achieved yet. Further studies are needed, since studying tea's compounds and mechanisms of action may yield new insights in developing new therapeutic strategies for the treatment of bladder cancer.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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LIST OF ABBREVIATIONS

BT	=	Black tea
BBN	=	N-butyl-N- (4-hydroxybutyl) nitrosamine
EC	=	Epicatechin
ECG	=	Epicatechin-3-gallate
EGC	=	Epigallocatechin
EGCG	=	Epigallocatechin-3-gallate
EGFR	=	Epidermal growth factor receptor
GT	=	Green tea
H_2O_2	=	Hydrogen peroxide
OT	=	Oolong tea
PO	=	Polyphenol oxidase
PP-60	=	Polyphenon-60
RNS	=	Reactive nitrogen species
ROS	=	Reactive oxygen species
pRb	=	Retinoblastoma protein
TCC	=	Transitional cell carcinoma
WT	=	White tea

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