Review paper

The black tea bioactivity: an overview

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Abstract

Tea is one of the most popular beverages in many countries and is the second, after the pure water, most consumed drink in the world, but consumption habit varies between different countries. Incidence of different diseases varies widely across the world and many investigators relate these differences to diet including habitual tea drinking. It is consumed mostly as green tea and black tea where other forms such oolong; red or white teas are less popular. Green tea was extensively investigated on its health benefits but black tea is only now catching the serious attention of scientific community. Compounds contained in black tea such as theaflavins and thearubigens contribute to black tea dark color and distinctive flavor. They also provide health benefits originally attributed solely to green tea. This review summarizes available information on bioactive ingredients of tea, their bioactivity and relation to diseases, bioavailability with special attention to health benefits of black tea.

Key words: *black tea, polyphenols, bioavailability, cancer, cardiovascular.*

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Tea is the second, after the pure water, most consumed drink in the world. It is one of the most well-liked beverages in many countries. Among other popular drinks it contains the highest concentrations of minerals and antioxidants. The real teas are produced from the same species of the plant *Camellia sinensis*. There are four types of basic teas: unfermented green tea, fully fermented black tea, red tea which is partially fermented oolong tea and white tea from tea leaf buds. Approximately 20% of the world's consumption is in the form of green tea, and other 80% are black and oolong teas [1]. Tea is grown in about 30 countries but as a popular drink it is mostly consumed in Asia and Europe, less in North America and North Africa (mainly Morocco). Green tea is popular in whole Asia while oolong is mostly drank in China and Taiwan [2].

Bioactive ingredients of fresh leaves and green tea

Fresh leaves of tea contain on average: 36% polyphenolic compounds, 25% carbohydrates (pectins, glucose, fructose, cellulose), 15% proteins, 6.5% lignin, 5% minerals and trace elements (magnesium, chromium, iron, copper, zinc, sodium, cobalt, potassium, etc.), 4% amino acids (such as theanine [5-*N*-ethyl-glutamine], glutamic acid, tryptophan, aspartic acid) 2% lipids, 1.5% organic acids, 0.5% chlorophyll as well as carotenoids and ethereal substances below 0.1%, vitamins (B, C, E) [3]. Green tea leaves are pan-fried or steamed, which prevents different bioactive compounds, like many polyphenolic flavonoids, from being oxidized. An average serving of green tea is brewed of 2 g of leaves in ~200 ml of hot water and contains approximately 600-900 mg of water extractable solids including tea catechins (30-40% by weight). The rich content of catechins representing approximately 90% of the polyphenolic fraction in green tea (considerably more than in black tea) is believed to generate its pro-health effects (Table 1). Tea catechins appear as isomers trans-catechins and cis-catechins depending on the stereochemical configuration of the 3',4'-dihydroxyphenyl and hydroxyl groups at the 2- and 3-positions of the C-ring [4]. Main catechins found in green tea are: catechin (C), gallocatechin (GC),

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epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG). In addition, green tea contains alkaloids (caffeine) and other flavonoids such as quercetin, kaempherol, and myricetin [5-7]. Epigallocatechin gallate was the most extensively studied active component of green tea and is known for its anti-cancer properties and potent antioxidative effect. It is a stronger antioxidant than either vitamin C or E.

Bioactive ingredients of black tea

There are various types of black tea, mostly named after the geographical region they came from. *Assam tea* is named after Assam, the region in India. It brews as burgundy red with rich aroma and a strong, malty taste. *Yunnan tea* is produced in Yunnan region of China. It is char-

Table 1. Content of different polyphenols in green and black tea (μg/ml) [13]

Compound	Green tea (μg/ml)	Black tea (μg/ml)
catechin (C)	21	20
(–)-epicatechin (EC)	98	37
(–)-epicatechin-3-gallate (ECG)	90	73
(–)-epigallocatechin (EGC)	411	42
(–)-epigallocatechin-3-gallate (EGCG)	444	128
Total catechins	**1064**	**300**
theaflavin (TF1)	0	22
theaflavin-3-gallate (TF2a)	0	20
theaflavin-3'-gallate (TF2b)	0	13
theaflavin-3,3'-digallate (TF3)	0	9
Total theaflavins	**0**	**64**

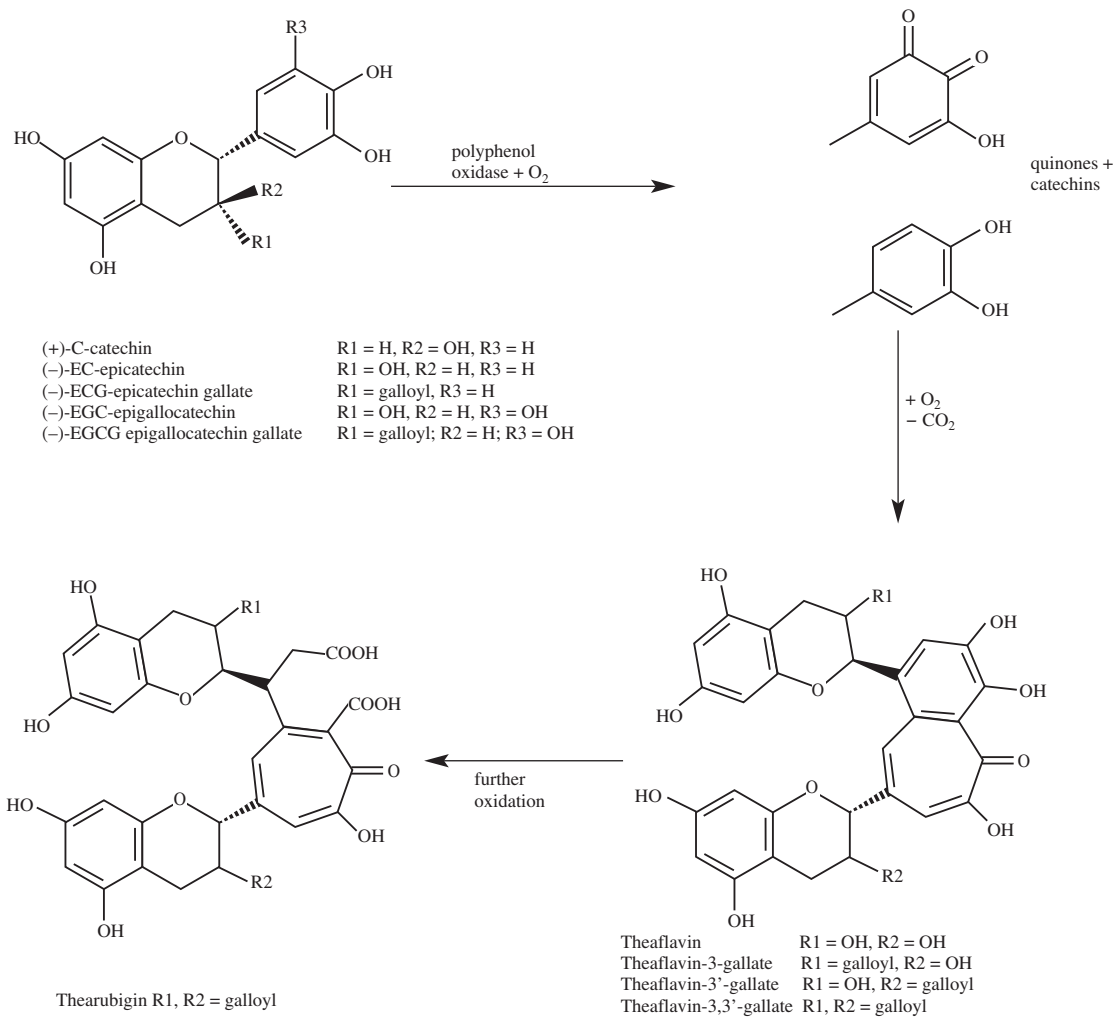

Fig. 1. Conversion of catechins of green tea to theaflavins and thearubigins of black tea [1]

acterized by malty and peppery flavor. Darjeeling tea coming from the Darjeeling region in West Bengal, India is famous for its it light-color floral aroma with tannic characteristics.

Nilgiri tea grows in the hills of the Nilgiri district of Tamil Nadu in India. It is very aromatic with a distinct briskness and a tantalizing flavor. *Ceylon tea* is cultivated in Sri Lanka (formerly Ceylon). It is known for its citrus taste and therefore is available in pure but also blended form [8, 9].

The tea fermentation process allows the leaves to undergo enzymatic oxidation when polyphenol oxidase causes polymerization of flavan-3-ols to catechin oligomers – bisflavanols, theaflavins, thearubigins and others. As a result only ~15% catechins from green tea remain unchanged, the rest is transformed to theaflavins and thearubigins [1] – see Fig. 1. Hence, in contrast to green tea with yellowish-greenish hues, fully fermented black tea has a dark brown hue and a sweet aroma of malt sugar.

The typical black tea brew is composed of number of small molecules, mostly alkaloids (e.g. theobromine and caffeine), carbohydrates and aminoacids (including theanine) as well as glycosylated flavonoids, together accounting for 30-40% of the dry weight. The remaining 60-70% consists of poorly characterized polyphenolic fermentation products, in that number oxytheotannins further subdivided into theaflavins and thearubigins. Theaflavins (a mixture of [theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin-3,3'-digallate] posses benzotropolone rings with dihydroxy or trihydroxy aromatic moieties as substituents and a characteristic yellow-orange color [10]. The red-brown or dark brown thearubigins consist of more than 5000 individual compounds retaining chiral properties of flavanols and theaflavins while prone to aggregation in aqueous solution. Their structures and bioavailability are still not well characterized [11]. Theaflavins and thearubigins account for 3-6% and 12-18% of dry weight of black tea and contribute to its strong, bitter flavor and characteristic dark color [12]. Comparison of a content of the basic compounds in crude black and green tea extract is given in Table 1 [13].

During the production process as much as 75% of catechins from tea leaves undergo oxidation and partial polymerization due to the enzymatic processing by polyphenol oxidase and other endogenous enzymes. Therefore, the final black tea composition considerably depends on the processing technology.

In average expected composition of black tea solid extract includes: catechins (10-12%), flavonols (6-8%), theaflavins (3-6%), thearubigins (12-18%), phenolic acids (10-12%), amino acids (13-15%), methylxanthines (8-11%), carbohydrates (15%), proteins (1%), and minerals (10%). The most important flavonols in black tea are myricetin, quercetin, kaempferol and ruthin, similar as in green tea. Black tea also contains phenolic acids, caffeine (about one third the amount typical for coffee) and amino acids including theanine (5-*N*-ethyl-glutamine) which occurs only in the tea leaves [3].

Theanine (γ-glutamylethylamine) is a compound unique for tea accounting for almost 50% of its aminoacid content as well as unique "brothy" taste. It is known for considerable neuroprotective effects and cognition enhancing properties, assists in brain function development i.e. central nervous system maturation [14]. Theanine was also shown to act as an neurotransmitter, modulator of serotonine and dopamine levels enhancing memory and learning abilities [15]. Importantly enough theanine plays its positive role in attentional processing in synergy with caffeine [16].

Black tea bioactivity – known facts

The impact of green tea, in particular its flavonoids and catechins, on the oxidant-antioxidant balance has been extensively studied. Their antioxidative properties are recognized and mostly attributed to the ability to inhibit free radical generation or biological activity activity as well as to chelate transition metal ions, mainly Fe and Cu catalyzing the free radical reactions.

On the other hand little is known about more complex compounds formed from catechins during natural fermentation in the process of black tea production [2, 17]. While catechins repreFsent ~90% of phenolic fraction in green tea, in black tea brew only ~15% of them remains not oxidized. Still, black tea is a valuable source of polyphenols, majority of them belonging to oxytheotannins, represented by theaflavins and thearubigins. Rechner *et al*. have shown that a cup of black tea delivers on average ~260 mg of polyphenols of which ~220 mg stand for theaflavins and thearubigins. Authors researched seven different brands of black tea marketed in England showing that each contained similar combination of polyphenols dominated by thearubigins (75-82% of total phenolics) and followed in a decreasing order by theaflavins, flavan-3-ols, flavonols, gallic acids and hydroxycinnamates [18]. Among them epigallocatechin gallate, four theaflavins, epicatechin gallate, quercetin-3-rutinoside, 4-caffeoylquinic acid were clearly and theogallin tentatively identified, with the total of 20 polyphenols as quantified by HPLC analysis. The antioxidant activity was assessed independently by three different methods (Trolox Equivalent Antioxidant Capacity – TEAC, Oxygen Radical Absorbence Capacity – ORAC, Ferric Ion Reducing Antioxidant Power – FRAP) showing good correlation in-between the methods as well as corresponding with previously published studies that characterized oxytheotannins as effective scavengers of free oxygen (superoxide anions, singlet oxygen) and nitric (nitric oxide, peroxynitrite) reactive oxygen species (ROS). Interestingly, theaflavins being dimers contain more hydroxyl (OH) groups than catechins, and this structural detail is pivotal for radical scavenging abilities. As a result, some

theaflavins, like TF3 reveal higher antioxidative activity than EGCG, the strongest antioxidant among catechins. Also, it was demonstrated that theflavins react over 10 times faster with superoxide radical than EGCG [3].

Beside free radicals scavenging theaflavins antioxidant properties are attributed to their ability to inhibit pro-oxidant enzymes and form stable complexes with iron or copper ions, therefore preventing free radical generation and lipid peroxidation. Yet, the concentration of black tea polyphenols in human blood even after considerable tea ingestion is 100-1000 times lower than the circulating amounts of other physiological antioxidants, like ascorbic acid or glutathione. That fact provokes the question about the practical meaning of the abovementioned antioxidant activity of black tea. Does it really induce any meaningful effects *in vivo*?

The increased antioxidant activity has been observed in laboratory animals exposed to the prolonged black tea enriched diet. In humans however, results were somewhat diverse. The *ex vivo* studies clearly confirmed the scavenging properties of black tea and catechins towards human cells. Interestingly, black tea extract in comparison to catechins exerted higher protective effect towards various types of oxidative stress [3, 19]. The ion chelating activity of tea polyphenols is a well proven fact. They avidly interact with iron ions forming the insoluble complexes, black tea more than the non-fermented green tea. This process inhibits haem absorption in the gastrointestinal tract, though involves the non-haem iron only and might be reversed in the presence of the ascorbic acid. It is not clear to what extend this phenomenon is responsible for the antioxidant activity of black tea in the real-life setting, however definitely affects the balanced iron turnover.

However, good quality data on the comparison of the antioxidative capacity of black and green tea are somewhat contradictory concluding that black tea activity is higher, on par or diminished in relation to green. Prior and Cao compared eighteen green and black tea brands from different sources demonstrating that an average antioxidative capacity (ORAC) and total phenolic content for the black tea is clearly higher than for green one [20]. Alternatively, Benzie and Szeto evaluated 25 blends of local Hong Kong tea by FRAP method concluding that green had the highest antioxidant potential while black and oolong tea were similar and less potent in that respect [21]. Still, when green tea was evaluated against black tea of the same brand (Assam from India) thus eliminating possible discrepancies due to the different plants, environmental factors and production site, no considerable differences in TEAC potential were observed [22]. Consequently, it should be accepted that ROS scavenging potential of black tea differs for different commercial brands, solidly depending on geography and technology of production. Thus, results from any clinical studies in humans strongly depend on local circumstances. Positive impact of black tea polyphenols on consumer health seriously depends on people overall metabolism but also bioavailability of their metabolites [18].

Xie *et al*. signalized that flavanols and other constituents of black tea in a pure form are not only strong antioxidants but also the effective lipoxygenase inhibitors [23]. It is worth noticing that human oxygenases are responsible for fatty acids peroxidation in a submembrane environment of cells, clearly related to ROS, ubiquitous in the body and implicated in many human diseases (inflammatory, cardiovascular, cancers, neurological disorders). Halder and Bhaduri studied black tea extract as possible preventative of oxidative stress provoked in human blood by different inducers. They have found that black tea or its chosen components could avert lipid peroxidation, degradation of membrane proteins and reverse membrane fluidity to full restoration of its architecture. In their experiments black tea extract was performing better than individual catechins proving that theaflavins and thearubigins which dominate in black tea brew are just as effective or better than smaller and simpler catechins [19].

Bioavailability of black tea components: problems and promises

Extensive data indicate that bioactive components of black tea might be quite important for prevention of considerable number of disorders. While abovementioned studies on black tea components seem to strongly support that view, their bioavailability analysis points at its possible flaws. The relatively high molecular weight, low internal activity, poor absorption, high rate of metabolisms, and rapid inactivation of metabolism products are considered the most important reasons for rather low bioavailability of black tea polyphenols. It is also affected by the fact that phenolic OH group tends to form large hydration shells [24]. On the other hand Rechner *et al.* elegantly proved that incubation of the black tea brew with the simulated gastric acids significantly increased content of the theaflavins (140%). It was attributed to the cleavage of the thearubigins at low pH into smaller and therefore more readily absorbed theaflavins [18]. Some authors have also suggested the possibility of the prolonged colonic metabolism of black tea polyphenols, theaflavins and thearubigins [25].

Warden *et al*. quantitatively evaluated bioavailability of black tea catechins in healthy volunteers demonstrating their peak in the plasma 5 hours after ingestion, with only 1.68% of their total intake present in the blood, urine and feces [26]. Catechins were present in the blood for 24 hrs after single intake of tea. Interestingly, several studies demonstrated different bioavailability of gallated and non-gallated forms, but their conclusions were contradictory [26-28]. In our opinion methodological issues and difficulties in exact theaflavin metabolites estimation might be responsible for those discrepancies. Detection of theaflavin metabolites with the modified A and C ring only, could not

be considered adequate and reliable measure of their bioavailability [28, 29].

However, Leenen *et al.* have shown that antioxidative potential of plasma after black tea ingestion was not different to that observed after green tea, irrespective of the dissimilar catechin levels, confirming the suggestion of their rapid absorption from the gastrointestinal tract [30]. Similarly, the theaflavin and thearubigin fractions of black tea strongly inhibited human recombinant sulfotransferase SULT1A1 and SULT1A3 isoforms representing liver and intestinal enzymatic activity therefore affecting mechanisms regulating absorption [31].

Still, the bioavailability of theaflavins and thearubigins and understanding of their metabolism and resulting metabolites is unclear and needs further cautious evaluation. Besides, little is known about possible effects of diet on the bioavailability of tea constituents. Immense differences in regional dietary habits are rarely considered whereas might be of considerable importance, responsible for example for varying result observed in clinical studies on the health effects of long-term ingestion of black tea. Similarly, the effect of milk added to the tea brew as it is customary in UK and other Commonwealth countries should be accounted for. While milk proteins bind directly and easily to black tea catechins and flavonols, Hertog *et al.* demonstrated that beneficial effect of black tea consumption on the risk of coronary heart disease was not observed in subjects consuming tea with milk [32]. It is still unclear what mechanism is responsible for this effect. Both *ex vivo* and *in vivo* studies are equivocal at best.

Consequently, any studies evaluating the *in vivo* bioactivity of black tea or its constituents should strictly define the studied group according to demographic (race) as well as geographic criteria. Both strongly imply critical differences in tea content (black vs. green, brands of black tea) as well as consumption habits (with milk pre-boiled or fresh, with lemon, habitual diet) that might considerably affect the outcomes. This particular fallacy is unfortunately quite common. If black tea undeniable bioactivity is to be evaluated properly and relevantly exploited in humans more well designed studies are needed.

Medicinal effects of black tea

Bioactive components of the black tea brew have been attracting much attention with regard to human health. While *in vitro* data demonstrate quite convincingly their considerable biological activity, animal and clinical studies have been less conclusive regarding to their effectiveness and potential applicability in disease prevention or therapy (supplementary role). Still, some data provide intriguing evidence on their beneficial effects, in particular in chronic pathologies characterized by high oxidative stress.

Atherosclerosis and cardiovascular diseases

The mechanism of atherosclerosis and consequently cardiovascular diseases (CVD) involves chronic inflammation, endothelial dysfunction and metabolic imbalance. Animal studies showed that black tea consumption reduces cholesterol liver synthesis and its serum levels thrice more effective than green tea [33, 34]. Similar effect was demonstrated by placebo-controlled randomized study in humans with five servings of black tea resulting in slight drop of total and LDL cholesterol in serum of mildly hipercholesterolemic patients [35]. Vermeer *et al*. proved as well that black tea theaflavins, in particular theaflavin-3-gallate, might interfere with intestinal cholesterol absorption [10]. In contrast, data on the low density lipoprotein (LDL) oxidation are unequivocal. Some authors observed no protective effect of black tea [36]. Others, as Ishikawa, documented that catechins, especially EGCG, and theaflavins strongly delay LDL oxidation i.e. atherosclerosis development and progression [37]. And finally some suggested that black tea might have greater impact on the *ex vivo* lipoprotein oxidation that green tea [38]. As described before black tea components, like flavonoids, are reducing agents capable to effectively chelate metals involved in cellular oxidation reactions. Hence their preventive/ameliorating effect on the oxidative stress within endothelial barrier supporting the normalization of endogenous vasodilators production, restoration of physiological endothelial barrier permeability as well as down-regulation of inflammatory markers and mediators expressed by endothelial cells. Jochmann *et al*. proven that beneficial effects of black tea on the endothelial function, both *ex vivo* and in patients, were comparable to green tea [39]. Also, catechins are able to incuce cell-cycle arrest and interact with growth factor to inhibit vascular smooth muscle proliferation, a key event in the development and progression of atherosclerosis [40]. In addition, considerable effect of black tea on the metabolic and inflammatory markers represented by uric acid (UA) and C-reactive protein (CRP) was observed in controlled studies [41].

Still, epidemiological studies attempting to analyze the link between black tea consumption and risk of cardiovascular death are inconclusive. Meta-analysis of ten cohort studies and seven case-control studies performed by Peters *et al.* in 2001 demonstrated considerable heterogeneity preventing reliable estimation of black tea effect on the stroke and coronary heart disease incidence. Still, occurrence of myocardial infarction has been shown to decrease by 11% (relative risk = 0.89) providing that at least three cups of black tea were consumed per day [42]. Heterogeneity of evaluated data revealed by this analysis was consistent with size of the analyzed studies (smaller tending toward significant effects) and their geographical origin. On the other hand, Huxley *et al.* in their meta-analysis of prospective

cohort studies observed a 20% risk reduction in coronary artery disease mortality in participants within a top tertile of flavonoid intake However, subsequent large cohort projects performed in European Welsh (n = 17 228) and Japanese groups (n = 76 979) demonstrated no significant association between black tea consumption and the risk of CVD incidence both in men and women [43, 44].

Some authors suggested that black tea polyphenols might exert positive effects in patients already treated due to CVD [45]. Duffy *et al.* analyzed both short- and long-term effects of black tea on the endothelial dysfunction in 50 patients with confirmed coronary artery disease and showed significant endothelium-dependent flow-mediated arterial dilation [46]. Similar effect was described in healthy volunteers [47]. Also, Hirata *et al.* have shown improved coronary flow velocity reserve as assessed by transthoracic Doppler echocardiography following acute black tea consumption [48].

Acute and chronic inflammation

Considerable evidence points at the anti-inflammatory bioactivity of the black tea polyphenols. It is warranted not only by their anti-oxidant properties. Tea polyphenols and EGCG in particular are able to selectively affect the production and/or bioactivity of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, IL-8) and mediators (iNOS, COX), mostly by modulating cellular signaling processes (NF-κB) [15]. Moreover, both black tea extract and three major black tea-derived catechins: epicatechin gallate, epigallocatechin and epigallocatechin gallate, were shown not only to suppress production of IL-1β by human leukocytes, but also to enhance synthesis of well-known anti-inflammatory cytokine IL-10 [49]. In the animal models of paw oedema black tea extracts significantly inhibited acute inflammatory response induced by number of physiological mediators (histamine, serotonin, prostaglandin) [50]. It has been repeatedly suggested that chronic persistent inflammation both low-grade as in atherosclerosis and more severe as chronic arthritis are to certain extend down-regulated by the black tea constituents. However, it should be emphasized that these effects were observed mainly at catechins concentrations hardly achievable in human plasma *in vivo*. Therefore the average black tea consumption is quite unlikely to exert considerable protective anti-inflammatory effect. The beneficial effect of black tea on the caries development and progression seems to be the exception. Black tea brew in standard, customary quantities appears to suppress salivary amylase activity, decrease tooth surface pH and therefore reduce the growth and virulence of periodontal microorganisms [24, 51]. Moreover, regular consumption of black tea affects dental bone density due to the higher fluoride content as the tea plant tends to accumulate fluoride from soil as well as due to other chemical tea compounds, caffeine and phytoestrogens, influence [52].

Cancer

There is a vast literature suggesting beneficial effects of regular green tea intake on the malignancies incidence in humans including: liver, lung, stomach, pancreatic, colon, breast, oral and prostate cancer [53-58]. Still, the thorough Cochrane meta-analysis of 51 studies with more than 1.6 million participants did not provide any firm conclusions. On the contrary, it was implied that data were insufficient and inconsistent. Still, in some types of neoplastic disease as prostate or lung cancer there was moderate evidence of risk reduction shown, while in others as gastric or urinary bladder cancer no effect was observed [1]. Recent exploratory meta-analysis of 13 observational studies confirmed borderline significant association between high green tea consumption and lower prostate cancer risk, with significant ameliorating effect observed in case-control studies (OR = 0.43) [59]. Still, it should be emphasized that regulatory authorities in Europe and USA are not as yet satisfied with abovementioned evidence. Green tea is not registered or officially recommended as an effective bioactive medicinal substance.

Epidemiological data concerning black tea effects are even more scant and generally suggest much weaker effect if any. In the citied meta-analysis no statistically significant association was observed between black tea consumption and cancer risk [59]. Numbers of studies provide data on the use of green tea or green tea polyphenols to enhance the effectiveness of chemo/radiotherapy. For black tea no compelling data are available [60]. While results of epidemiological analyses are irregular, research data provide quite persuasive evidence supporting chemo preventive effect of teas, though much stronger for green than for black tea. There are multiple and quite divergent mechanisms proposed to explain anti-cancerous tea effects including antioxidant, anti-proliferative, anti-inflammatory, antibactericidal and antiviral activity. Many of them are attributed to the polyphenols bioactivity, EGCG in particular. Strong radical scavengers and metal chelators exert ameliorating effect on the oxidative stress, one of the driving processes in cancerogenesis [3]. Interestingly, polyphenols induce also production of endogenous anti-oxidant molecules and enzymes enhancing physiological defense mechanisms [61]. Gutierrez-Orozco *et al.* showed that both, green and black tea extracts exerted down-regulatory effect on the IL-8 production and secretion by IL-1β stimulated gastric cancer cells [62]. Therefore, it was demonstrated that both teas possess anti-inflammatory activity, also toward persistently acute inflammation. Observed effect was irrespective of their different catechin profiles and mediated via inhibition of intracellular transcription factor NF-κB [62]. In this context, anti-bacterial and anti-viral properties of green and black tea flavonoids should be recalled as they provide additional defensive anti-inflammatory mechanism potentially important in the real-life setting [24]. An excellent exam-

ple might be the abovementioned protective influence on caries as well as oral cancer cells development and progression [51, 63].

Tea extracts and constituents are also known for they ability to directly affect mutagenesis as well as cancer growth. The antimutagenic effect is well documented for the green but also black tea polyphenols, in particular theaflavins and thearubigins and attributed both to the inhibition of the oxidative DNA damage as well as modulation of xenobiotic-metabolizing enzymes [63, 64]. Also, considerable literature demonstrates their inhibitory effect on the tumor cell proliferation both *in vitro* and *in vivo* in animal models. Lyn-Cook *et al.* showed that green and black tea polyphenols, black tea theaflavins and pure EGCG decreased down to 10% proliferative rate of pancreatic (HPAC) and prostate tumor (LNCaP) cells [65]. Similar effect of black tea theaflavins was observed in the mice model of lung cancer [66]. It was also proven that tea polyphenols considerably suppress production and reactivity of cytokines regulating tumor cells growth, like VEGF, PDGF or protein kinases, like MAP kinase or IkB kinase. Also, Zhao *et al.* demonstrated that black tea aqueous extract, predominantly theabrownins, not only significantly decreased gastric cells viability, but also induced cell cycle arrest in S phase. Interestingly, normal gastric cells were not affected [67]. In addition, considerable effect on the cancer cells apoptosis was shown, though considerably stronger for green tea most probably due to the higher catechin content. It was suggested that tea polyphenols effectively affect these processes as well mainly via regulation of nuclear factor κB, Akt and p53 [68, 69]. Hibasami *et al.* shown that black tea theaflavins can induce apoptosis and inhibit the growth of human stomach cancer cells in a time and dose dependent manner [70]. Finally, green and black tea extracts proved to suppress cancer cells invasiveness and ability to metastasize via inhibition of angiogenesis, proteases and cytokines production [71].

Still, irrespective of numerous studies analyzing in detail the mechanism of tea biological activity, there is considerable inconsistency between epidemiological and experimental data. Several factors might be responsive for that including inadequate average tea consumption (to low intake of bioactive tea constituents to exert positive effect). However, human genetic heterogeneity together with cultural and life-style differences are most probably the key reasons. Described dissimilarities between European and Asian cohort studies strongly support that suggestion.

Other disorders

It was implied that antioxidant and anti-inflammatory effects of tea consumption might be useful to support pharmacological therapy of neurodegenerative Parkinson's and Alzheimer's disorders by attenuating the degeneration of dopamine neurons and promote neurons survival [72, 73]. In recent years number of experimental and observational studies in humans provided strong evidence for the positive effect of green and black teas on weight control in obese subjects [73-77]. Most likely, the underlying mechanism is very complex and still not very well understood. Still, the significant reduction of serum cholesterol both in experimental animals and humans was observed [78]. Uchiyama *et al.* have shown that irrespective of metabolism modification black tea polyphenols interfere with intestinal lipids absorption [79]. Meanwhile, Chen *et al.* suggested their positive effect on metabolic gene expression, glucose tolerance and body composition in animals fed a high-fat diet [74]. Still, human interventional studies are positive for green tea only and mostly its extracts. Interestingly, positive interaction between tea and physical exercise in abdominal fat and serum lipids control has been suggested [75].

In summary, available epidemiologic and experimental studies showed the positive relationship between black tea consumption and prevention or possible cure of variety of diseases. However, more dose-response and mechanistic studies are needed to understand the effects of tea consumption on human ailments. Furthermore, appropriate strategies are warranted for future clinical trials transliterating animal data and small human experiments to fully proven nutraceutical.

References

1. Boehm K, Borrelli F, Ernst E, et al. (2009): Green tea (Camellia sinensis) for the prevention of cancer. Cochrane Database Syst Rev: (3) CD005004.
2. McKay DL, Blumberg JB (2002): The role of tea in human health: an update. J Am Coll Nutr 21: 1-13.
3. Łuczaj W, Skrzydlewska E (2005): Antioxidative properties of black tea. Prev Med 40: 910-918.
4. Friedman M, Mackey BE, Kim HJ, et al. (2007): Structure-activity relationships of tea compounds against human cancer cells. J Agric Food Chem 55: 243-253.
5. Graham HN (1992): Green tea composition, consumption, and polyphenol chemistry. Prev Med 21: 334-350.
6. Henning SM, Niu Y, Lee NH, et al. (2004): Bioavailability and antioxidant activity of tea flavanols after consumption of green tea, black tea, or a green tea extract supplement. Am J Clin Nutr 80: 1558-1564.
7. Crozier A, Jaganath IB, Clifford MN (2009): Dietary phenolics: chemistry, bioavailability and effects on health. Nat Prod Rep 26: 1001-1043.
8. Banerjee H, Ganguly P, Roy S, et al. Persistence and safety risk assessment of propineb in Indian tea. Environ Monit Assess 170: 311-314.
9. Dutta R, Kashwan KR, Bhuyan M, et al. (2003): Electronic nose based tea quality standardization. Neural Netw 16: 847-853.
10. Vermeer MA, Mulder TP, Molhuizen HO (2008): Theaflavins from black tea, especially theaflavin-3-gallate, reduce the incorporation of cholesterol into mixed micelles. J Agric Food Chem 56: 12031-12036.

11. Kuhnert N (2010): Unraveling the structure of the black tea thearubigins. Arch Biochem Biophys 501: 37-51.
12. Menet MC, Sang S, Yang CS, et al. (2004): Analysis of theaflavins and thearubigins from black tea extract by MALDI-TOF mass spectrometry. J Agric Food Chem 52: 2455-2461.
13. Kuroda Y, Hara Y (1999): Antimutagenic and anticarcinogenic activity of tea polyphenols. Mutat Res 436: 69-97.
14. Yamada T, Terashima T, Wada K, et al. (2007): Theanine, r-glutamylethylamide, increases neurotransmission concentrations and neurotrophin mRNA levels in the brain during lactation. Life Sci 81: 1247-1255.
15. de Mejia EG, Ramirez-Mares MV, Puangpraphant S (2009): Bioactive components of tea: cancer, inflammation and behavior. Brain Behav Immun 23: 721-731.
16. Kelly SP, Gomez-Ramirez M, Montesi JL, Foxe JJ (2008): L-theanine and caffeine in combination affect human cognition as evidenced by oscillatory alpha-band activity and attention task performance. J Nutr 138: 1572S-1577S.
17. Leung LK, Su Y, Chen R, et al. (2001): Theaflavins in black tea and catechins in green tea are equally effective antioxidants. J Nutr 131: 2248-2251.
18. Rechner AR, Wagner E, Van Buren L, et al. (2002): Black tea represents a major source of dietary phenolics among regular tea drinkers. Free Radic Res 36: 1127-1135.
19. Halder J, Bhaduri AN (1998): Protective role of black tea against oxidative damage of human red blood cells. Biochem Biophys Res Commun 244: 903-907.
20. Prior RL, Cao G (1999): Antioxidant capacity and polyphenolic components of teas: implications for altering *in vivo* antioxidant status. Proc Soc Exp Biol Med 220: 255-261.
21. Benzie IF, Szeto YT (1999): Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. J Agric Food Chem 47: 633-636.
22. Schlesier K, Harwat M, Böhm V, Bitsch R (2002): Assessment of antioxidant activity by using different *in vitro* methods. Free Radic Res 36: 177-187.
23. Xie B, Shi H, Chen Q, Ho CT (1993): Antioxidant properties of fractions and polyphenol constituents from green, oolong and black teas. Proc Natl Sci Counc Repub China B 17: 77-84.
24. Friedman M (2007): Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. Mol Nutr Food Res 51: 116-134.
25. Clifford MN, Copeland EL, Bloxsidge JP, Mitchell LA (2000): Hippuric acid as a major excretion product associated with black tea consumption. Xenobiotica 30: 317-326.
26. Warden BA, Smith LS, Beecher GR, et al. (2001): Catechins are bioavailable in men and women drinking black tea throughout the day. J Nutr 131: 1731-1737.
27. Lee MJ, Wang ZY, Li H, et al. (1995): Analysis of plasma and urinary tea polyphenols in human subjects. Cancer Epidemiol Biomarkers Prev 4: 393-399.
28. Mulder TP, van Platerink CJ, Wijnand Schuyl PJ, van Amelsvoort JM (2001): Analysis of theaflavins in biological fluids using liquid chromatography-electrospray mass spectrometry. J Chromatogr B Biomed Sci Appl 760: 271-279.
29. Wiseman S, Mulder T, Rietveld A (2001): Tea flavonoids: bioavailability *in vivo* and effects on cell signaling pathways *in vitro*. Antioxid Redox Signal 3: 1009-1021.
30. Leenen R, Roodenburg AJ, Tijburg LB, et al. (2000): A single dose of tea with or without milk increases plasma antioxidant activity in humans. Eur J Clin Nutr 54: 87-92.
31. Nishimuta H, Ohtani H, Tsujimoto M, et al. (2007): Inhibitory effects of various beverages on human recombinant sulfotransferase isoforms SULT1A1 and SULT1A3. Biopharm Drug Dispos 28: 491-500.
32. Hertog MG, Sweetnam PM, Fehily AM, et al. (1997): Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. Am J Clin Nutr 65: 1489-1494.
33. Singh DK, Banerjee S, Porter TD (2009): Green and black tea extracts inhibit HMG-CoA reductase and activate AMP kinase to decrease cholesterol synthesis in hepatoma cells. J Nutr Biochem 20: 816-822.
34. Riemersma RA, Rice-Evans CA, Tyrrell RM, et al. (2001): Tea flavonoids and cardiovascular health. QJM 94: 277-282.
35. Davies MJ, Judd JT, Baer DJ, et al. (2003): Black tea consumption reduces total and LDL cholesterol in mildly hypercholesterolemic adults. J Nutr 133: 3298S-3302S.
36. McAnlis GT, McEneny J, Pearce J, Young IS (1998): Black tea consumption does not protect low density lipoprotein from oxidative modification. Eur J Clin Nutr 52: 202-206.
37. Ishikawa T, Suzukawa M, Ito T, et al. (1997): Effect of tea flavonoid supplementation on the susceptibility of low-density lipoprotein to oxidative modification. Am J Clin Nutr 66: 261-266.
38. Hodgson JM, Puddey IB, Burke V, et al. (1999): Effects on blood pressure of drinking green and black tea. J Hypertens 17: 457-463.
39. Jochmann N, Lorenz M, Krosigk A, et al. (2008): The efficacy of black tea in ameliorating endothelial function is equivalent to that of green tea. Br J Nutr 99: 863-868.
40. Stangl V, Dreger H, Stangl K, Lorenz M (2007): Molecular targets of tea polyphenols in the cardiovascular system. Cardiovasc Res 73: 348-358.
41. Bahorun T, Luximon-Ramma A, Gunness TK, et al. (2010): Black tea reduces uric acid and C-reactive protein levels in humans susceptible to cardiovascular diseases. Toxicology 278: 68-74.
42. Peters U, Poole C, Arab L (2001): Does tea affect cardiovascular disease? A meta-analysis. Am J Epidemiol 154: 495-503.
43. Sesso HD, Paffenbarger RS Jr, Oguma Y, Lee IM (2003): Lack of association between tea and cardiovascular disease in college alumni. Int J Epidemiol 32: 527-533.
44. Mineharu Y, Koizumi A, Wada Y, et al. (2011): Coffee, green tea, black tea and oolong tea consumption and risk of mortality from cardiovascular disease in Japanese men and women. J Epidemiol Community Health 65: 230-240.
45. Widlansky ME, Duffy SJ, Hamburg NM, et al. (2005): Effects of black tea consumption on plasma catechins and markers of oxidative stress and inflammation in patients with coronary artery disease. Free Radic Biol Med 38: 499-506.
46. Duffy SJ, Keaney JF Jr, Holbrook M, et al. (2001): Short- and long-term black tea consumption reverses endothelial dysfunction in patients with coronary artery disease. Circulation 104: 151-156.
47. Grassi D, Mulder TP, Draijer R, et al. (2009): Black tea consumption dose-dependently improves flow-mediated dilation in healthy males. J Hypertens 27: 774-781.
48. Hirata K, Shimada K, Watanabe H, et al. (2004): Black tea increases coronary flow velocity reserve in healthy male subjects. Am J Cardiol 93: 1384-1388.
49. Crouvezier S, Powell B, Keir D, Yaqoob P (2001): The effects of phenolic components of tea on the production of pro- and

anti-inflammatory cytokines by human leukocytes *in vitro*. Cytokine 13: 280-286.
50. Roy DK, Kumar KT, Karmakar S, et al. (2008): Pharmacological studies on Indian black tea (leaf variety) in acute and chronic inflammatory conditions. Phytother Res 22: 814-819.
51. Lee MJ, Lambert JD, Prabhu S, et al. (2004): Delivery of tea polyphenols to the oral cavity by green tea leaves and black tea extract. Cancer Epidemiol Biomarkers Prev 13: 132-137.
52. Gardner EJ, Ruxton CH, Leeds AR (2007): Black tea – helpful or harmful? A review of the evidence. Eur J Clin Nutr 61: 3-18.
53. Chen D, Milacic V, Chen MS, et al. (2008): Tea polyphenols, their biological effects and potential molecular targets. Histol Histopathol 23: 487-496.
54. Le Marchand L (2002): Cancer preventive effects of flavonoids – a review. Biomed Pharmacother 56: 296-301.
55. Tang N, Wu Y, Zhou B, et al. (2009): Green tea, black tea consumption and risk of lung cancer: a meta-analysis. Lung Cancer 65: 274-283.
56. Wang P, Aronson WJ, Huang M, et al. (2010): Green tea polyphenols and metabolites in prostatectomy tissue: implications for cancer prevention. Cancer Prev Res (Phila) 3: 985-993.
57. Zhang M, Holman CD, Huang JP, Xie X (2007): Green tea and the prevention of breast cancer: a case-control study in Southeast China. Carcinogenesis 28: 1074-1078.
58. Kurahashi N, Sasazuki S, Iwasaki M, et al. (2008): Green tea consumption and prostate cancer risk in Japanese men: a prospective study. Am J Epidemiol 167: 71-77.
59. Henning SM, Wang P, Heber D (2011): Chemopreventive effects of tea in prostate cancer: green tea versus black tea. Mol Nutr Food Res 55: 905-920.
60. Yamamoto T, Hsu S, Lewis J, et al. (2003): Green tea polyphenol causes differential oxidative environments in tumor versus normal epithelial cells. J Pharmacol Exp Ther 307: 230-236.
61. Lambert JD, Elias RJ (2010): The antioxidant and pro-oxidant activities of green tea polyphenols: a role in cancer prevention. Arch Biochem Biophys 501: 65-72.
62. Gutierrez-Orozco F, Stephens BR, Neilson AP, et al. (2010): Green and black tea inhibit cytokine-induced IL-8 production and secretion in AGS gastric cancer cells via inhibition of NF-κB activity. Planta Med 76: 1659-1665.
63. Letchoumy PV, Mohan KV, Stegeman JJ, et al. (2008): *In vitro* antioxidative potential of lactoferrin and black tea polyphenols and protective effects *in vivo* on carcinogen activation, DNA damage, proliferation, invasion, and angiogenesis during experimental oral carcinogenesis. Oncol Res 17: 193-203.
64. Gupta S, Chaudhuri T, Seth P, et al. (2002): Antimutagenic effects of black tea (World Blend) and its two active polyphenols theaflavins and thearubigins in Salmonella assays. Phytother Res 16: 655-661.
65. Lyn-Cook BD, Rogers T, Yan Y, et al. (1999): Chemopreventive effects of tea extracts and various components on human pancreatic and prostate tumor cells *in vitro*. Nutr Cancer 35: 80-86.
66. Yang GY, Liu Z, Seril DN, et al. (1997): Black tea constituents, theaflavins, inhibit 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumorigenesis in A/J mice. Carcinogenesis 18: 2361-2365.
67. Zhao H, Zhang M, Zhao L, et al. (2011): Changes of constituents and activity to apoptosis and cell cycle during fermentation of tea. Int J Mol Sci 12: 1862-1875.
68. Kaur S, Greaves P, Cooke DN, et al. (2007): Breast cancer prevention by green tea catechins and black tea theaflavins in the C3(1) SV40 T,t antigen transgenic mouse model is accompanied by increased apoptosis and a decrease in oxidative DNA adducts. J Agric Food Chem 55: 3378-3385.
69. George J, Singh M, Srivastava AK, et al. (2011): Resveratrol and black tea polyphenol combination synergistically suppress mouse skin tumors growth by inhibition of activated MAPKs and p53. PLoS One 6: e23395.
70. Hibasami H, Komiya T, Achiwa Y, et al. (1998): Black tea theaflavins induce programmed cell death in cultured human stomach cancer cells. Int J Mol Med 1: 725-727.
71. Larsen CA, Dashwood RH, Bisson WH (2010): Tea catechins as inhibitors of receptor tyrosine kinases: mechanistic insights and human relevance. Pharmacol Res 62: 457-464.
72. Hamaguchi T, Ono K, Murase A, Yamada M (2009): Phenolic compounds prevent Alzheimer's pathology through different effects on the amyloid-beta aggregation pathway. Am J Pathol 175: 2557-2565.
73. Skrzypczak-Jankun E, Jankun J (2010): Theaflavin digallate inactivates plasminogen activator inhibitor: could tea help in Alzheimer's disease and obesity? Int J Mol Med 26: 45-50.
74. Chen N, Bezzina R, Hinch E, et al. (2009): Green tea, black tea, and epigallocatechin modify body composition, improve glucose tolerance, and differentially alter metabolic gene expression in rats fed a high-fat diet. Nutr Res 29: 784-793.
75. Grove KA, Lambert JD (2010): Laboratory, epidemiological, and human intervention studies show that tea (Camellia sinensis) may be useful in the prevention of obesity. J Nutr 140: 446-453.
76. Klaus S, Pültz S, Thöne-Reineke C, Wolfram S (2005): Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. Int J Obes (Lond) 29: 615-623.
77. Grove KA, Lambert JD (2010): Laboratory, epidemiological, and human intervention studies show that tea (Camellia sinensis) may be useful in the prevention of obesity. J Nutr 140: 446-453.
78. Matsumoto N, Okushio K, Hara Y (1998): Effect of black tea polyphenols on plasma lipids in cholesterol-fed rats. J Nutr Sci Vitaminol (Tokyo) 44: 337-342.
79. Uchiyama S, Taniguchi Y, Saka A, et al. (2011): Prevention of diet-induced obesity by dietary black tea polyphenols extract *in vitro* and *in vivo*. Nutrition 27: 287-292.