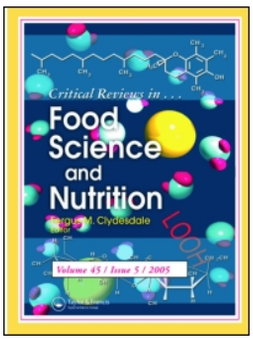

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Black tea: Phytochemicals, cancer chemoprevention, and clinical studies

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ABSTRACT

Tea (Camellia sinensis L.) is the most popular, flavored, functional, and therapeutic non-alcoholic drink consumed by two-thirds of the world's population. Black tea leaves are reported to contain thousands of bioactive constituents such as polyphenols, amino acids, volatile compounds, and alkaloids that exhibit a range of promising pharmacological properties. Due to strong antioxidant property, black tea inhibits the development of various cancers by regulating oxidative damage of biomolecules, endogenous antioxidants, and pathways of mutagen and transcription of antioxidant gene pool. Regular drinking of phytochemicals-rich black tea is linked to regulate several molecular targets, including COX-2, 5-LOX, AP-1, JNK, STAT, EGFR, AKT, Bcl2, NF-κB, Bcl-xL, caspases, p53, FOXO1, TNFα, PARP, and MAPK, which may be the basis of how dose of black tea prevents and cures cancer. In vitro and preclinical studies support the anti-cancer activity of black tea; however, its effect in human trails is uncertain, although more clinical experiments are needed at molecular levels to understand its anti-cancer property. This review discusses the current knowledge on phytochemistry, chemopreventive activity, and clinical applications of black tea to reveal its anti-cancer effect.

KEYWORDS

Black tea; phytochemistry; antioxidant; cancer prevention; anti-mutagenic

Abbreviations: AMPK, AMP-activated protein kinase; AOM, Azoxymethane; AP-1, Activated protein-1; B(a)P, Benzo [a] pyrene; Bcl2, B-cell lymphoma-2; Bcl-xL, B-cell lymphoma-extra large; CAT, Catalase; CI, Confidence interval; COX-2, Cyclooxygenase-2; CP, Cyclophosphamide; CYP, Cytochrome; DMBA, 7,12 Dimethyl benz(a) anthracene; DMH, 1,2-Dimethyl hydrazine; EAC, Ehrlich's ascites carcinoma; EC, (−)-Epicatechin; ECG, (−)-Epicatechin gallate; EGC, (−)-Epigallocatechin; EGCG, (−)-Epigallocatechin gallate; EGFR, Epidermal growth factor receptor; FRs, Free radicals; GC, (+)-Gallocatechin; GPx, Glutathione peroxidase; GR, Glutathione reductase; GSH, Glutathione; GST, Glutathione-S-transferase; HAAs, Heterocyclic aromatic amines; HUVECs, Human vascular endothelial cells; IGF-1, Insulin-like growth factor-1; IL-6, Interleukin-6; IQ, 2-Amino 3-methyl imidazo (4, 5-f) quinoline; JNK, c-Jun N-terminal kinase; LOX, Lipoxygenase; LPO, Lipid peroxidation; MAPK, Mitogen-activated protein kinases; MeIQx, 2-Amino-3,8-dimethylimidazo [4,5-f] quinoxaline; MNNG, *N*-Methyl-*N*′-nitro-*N*-nitrosoguanidine; mTOR, Mammalian target of rapamycin; NDEA, *N*-Dinitroso-diethyl amine; NF-κB, Nuclear factor-kappa B; PARP, Poly(ADP-ribose) polymerase; PhIP, 2-Amino1-methyl-6-phenylimidazo[4,5-b]pyridine; PI3K, Phosphoinositide 3-kinase; PO, Peroxidase; PPO, Polyphenol oxidase; PTP1B, Protein tyrosine phosphatase 1B; RR, Relative risks; SOD, Superoxide dismutase; STAT, Signal transducer and activator of transcription; TF1, Theaflavin; TF2, Theaflavin 3-*O*-gallate; TF3, Theaflavin 3′-O-gallate; TF4, Theaflavin 3,3′-O,O-digallate; TRs, Thearubigins

Introduction

Tea (*Camellia sinensis* L.) is the second most consumed flavored, functional, and therapeutic non-alcoholic beverage in the world, next to the water (Singh et al., 2011b; Hayat et al., 2013). The harvested shoots comprise an apical bud and two to three tender leaves containing 10–30% polyphenolic compounds (Li et al., 2013). The simple act of putting tea leaves into hot water has provided ancient societies with a tasty beverage associated with the observation of certain medicinal effects. Strong antioxidant activity of tea can be attributed to polyphenolic compounds (Gardner et al., 2007; Butt et al., 2014), which neutralize free radicals (FRs) that can damage the body's cells, leading to disease development (Chen et al., 2004; Singh et al., 2009c).

Polyphenols, particularly catechins, such as (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (EGCG), determine the quality of green tea leaves (Sharma and Rao, 2009; Singh et al., 2011b; Singh and Katiyar, 2013). Biotransformation of catechins during processing of leaves is usually divided into three basic types: black (fully oxidized), oolong (semi-oxidized), and green (non-oxidized). Of the total amount of tea produced and consumed in the world, 78% is black, 20% is green, and <2% is oolong tea. Black tea is consumed primarily in Western countries and in some Asian countries, whereas green tea is consumed primarily in India, China, Japan, and a few countries of North Africa and Middle East. During the mechanical

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maceration of green tea leaves, the oxidative polymerization and condensation of catechins into theaflavins (TFs) and thearubigins (TRs) takes place by the action of polyphenol oxidase (PPO) and peroxidase (PO) (Li et al., 2013). TFs and TRs could be the chemical entities not only responsible for the color of black tea brew but also accountable for a range of health benefits (Blot et al., 1997; Sharma and Rao, 2009).

Epidemiologic studies have also shown that black tea or its chemical constituents inhibit the growth of established cancers at various organ sites such as prostate, pancreas, liver, colon, and oral (Blot et al., 1997; Beltz et al., 2006). Black tea contains various groups of chemicals, including flavonoids (TFs, TRs, and catechins), phenolic acids (gallic, cauramic, caffeic, and chlorogenic acids), amino acids (theanine), methylxanthines (caffeine), proteins, lipids, carbohydrates, volatile compounds fluoride, β-carotene, and traces of vitamins A, K, C, and folate (Hayat et al., 2013; Li et al., 2013). A myriad of scientific data have shown that the pharmacological effects of black tea are mainly attributed to its polyphenolic compounds (Butt et al., 2014). Among them, TFs and TRs are the major polyphenols that appear to be the most potential antioxidants with respect to regulating aging, cell proliferation, and apoptosis in human cancer cell lines (Beltz et al., 2006; Henning et al., 2011; Li et al., 2013). In addition to therapeutic effects, TRs, TFs, and theanine are prime quality attributes of black tea, and contribute color and taste. Apart from green tea, black tea is currently a hot topic worldwide in both nutritional and therapeutic research because of the presence of key therapeutic TFs and TRs that are more bio-stable and direct-acting than those found in other plants (Li et al., 2013). The activities of these compounds are so all pervading that they are virtually broad spectrum in their actions. This paper provides a critical review on phytochemical constituents, anticancer property, and clinical applications of black tea and its bioactive compounds.

Chemical constituents

Polyphenolic compounds

Both green tea and black tea have similar chemical compositions, the basic difference between the two being the chemical changes that take place during their production. Polyphenols in tea mainly include the following six groups of compounds: flavanols, hydroxyl-4-flavanols, anthocyanins, flavones, flavonols, and phenolic acids (Drynan et al., 2010a; Hayat et al., 2013). Polyphenols contribute to the overall significant bitterness, astringency, and sweet taste, and constitute 16−30% of green tea, 3−10% of black tea, and 8−20% of oolong tea (Li et al., 2013). The main bioactive constituents of black tea belong to the polyphenol group accounting for 25–35% on the dry weight basis (Table 1). Black tea, as typically brewed in India, contains about 200-mg flavonoids per cup. Clinical investigations have documented a significant association between regular intake of black tea (>3 cups/day) and a reduced risk of diseases. It was associated with the presence of polyphenols (Li et al., 2013). TFs, TRs, theaflavinic acids, and TF isomers attribute taste, color tone, and body to the brew. Figure 1 illustrates the formation of TFs. The basic skeleton of TFs is benzotropolone, a bicyclic ring containing the tropolone structure. In contrast to the theoretical eight or more TFs that can be produced proportionally, usually there are four major TFs, including TF1, TF-3-O-gallate (TF2), TF-3′-O-gallate (TF3), and TF-3,3′-O,O-digallate (TF4) formed from catechins (Li et al., 2013; Butt et al., 2014). The benzotropolone (core of TF) is formed by the reaction of catechol and pyrogallol. The reaction between a catechol and a pyrogallolyl group on a gallate occurs at a slow rate and yields only small amounts of other TF isomers.

Isomers of TFs, such as iso-TF, neo-TF, theaflavate A−B, iso-TF-3′-O-gallate, neotheaflavate-3-O-gallate, theaflavic acids, theaflagallins, and methylated TFs, have also been isolated from black tea (Table 1; Fig. 1). The coupling between EC and (+)-gallocatechin (GC) forms iso-TF. Due to the trace amount of GC in green tea, the total concentration of iso-TF in black tea is normally not detectable and has not been reported to have any contribution to pharmacological activities. Similarly, iso-TF-3′-O-gallate was formed through reaction between ECG and GC and characterized in green tea (Li et al., 2013). Neo-TF-3-O-gallate has been isolated from black tea extract, and structurally identified by nuclear magnetic resonance (NMR) and mass spectrometry (MS). The structural difference between iso-TF and neo-TF is the orientation of benzotropolone core, which is reversed. Theaflagallins are enzymatic oxidative products of the reaction between catechins and pyrogallol (Sharma and Rao, 2009). The pyrogallol mainly comes from gallic acid in green tea or ester cleavage of gallates during the oxidation process. However, other authors confirmed that theaflagallins are theaflagallin and epitheaflagallin-3-O-gallate. Theaflavic acids and theaflavates have been found in some black tea infusions or extracts. In a model study of tea fermentation, epitheaflavic acid and epitheaflavic acid-3-Ogallate were formed and identified. There is possibility of their presence in industrial black tea manufacturing, because epicatechin and gallic acid are their precursors existing in green tea leaves (Li et al., 2013). However, these TF isomers, namely iso-TFs, neo-TFs, theaflagallins, theaflavates, and theaflavic acids, are usually trace components of black tea (Hayat et al., 2013). Recently, Chen et al. (2012) identified TF trigallate and tetragallate in black tea extract by liquid chromatography (LC)/electrospray ionization mass spectrometry.

Theaflavins are orange-yellow pigments that constitute ~0.5–2.0% of dry weight, depending upon the type of manufacture of black tea. Yet, TFs and TRs have more difficult to be identified than catechins, but they exhibit strong antiradical property. Black tea is equal to green tea in terms of antioxidant potential (Sharma and Rao, 2009). The uptake of TFs is relatively low compared with catechins, but this may be masked by imprecise methodologies that do not detect all of the TF metabolites (Hayat et al., 2013). The drinking of three cups of tea per day for two weeks (@ 2-g dry tea/cup) enhanced the concentration of flavonoids in the blood by 25%. The gallated flavonols are related to astringency and bitterness of black tea, while the non-gallated tea flavonols are associated with bitterness. Among the TFs, theoflavin is less astringent. The contribution of astringency by TF4 and TF3 was reported to be 6.4 and 2.2 times, respectively, to that of TF. The attractive color of tea infusion is also due to the presence of TFs, and it emerges as an important quality index of black tea.

On other hand, TRs are orange-brown colored compounds constituting about 6–18% of dry weight (Table 1). TR pigments contribute around 35% of the total color and provide brown

Table 1. Composition of tea and their impact on taste and color of brew.

Chemical constituents	Molecular formula	Molecular weight	% Dry weight		Taste	Taste threshold level (mol/L)	Color of brew
			Green tea	Black tea			
Total polyphenols			30–40	25–30			
Simple polyphenols			2	3			
Oxidized polyphenols			4–6	23–25			
Flavanols			20–30	Small amount	Astringent and bitter		Light yellow
(−)-Epicatechin (EC)	$C_{15}H_{14}O_6$	290	1–3				
(−)-Epicatechin gallate (ECG)	$C_{22}H_{18}O_{10}$	442	3–6				
(−)-Epigallocatechin (EG)	$C_{15}H_{14}O_7$	306	3–6				
(−)-Epigallocatechin gallate (EGCG)	$C_{22}H_{18}O_{11}$	458	9–13	0.5–0.9		190	
(+)-Catechin	$C_{15}H_{14}O_6$	290	1–2		Puckering astringent	410	
Flavonols and flavonol glucosides					Velvety astringent, mouth drying/ coating		
Quercetin	$C_{15}H_{10}O_7$	302	Trace	Trace			
Kaempferol	$C_{15}H_{10}O_6$	286	Trace	Trace			
Quercetin 3-rhamnoglucoside (Rutin)	$C_{27}H_{30}O_{16}$	610	0.9–1.0	Trace		0.001	Light yellow
Kaempferol 3-rhamnoglucoside	$C_{27}H_{30}O_{15}$	594	Trace	Trace		0.25	
Quercetin 3-rhamnodiglucoside	$C_{33}H_{40}O_{21}$	772	Trace	Trace		1.36	
Kaempferol 3-rhamnodiglucoside	$C_{33}H_{40}O_{21}$	756	Trace	Trace		0.67	
Phenolic acids					Astringent		
Theogallin	$C_{14}H_{15}O_{10}$	343	1.0	1.0			
Ellagic acid	$C_{14}H_6O_8$	302	Trace	Trace			
Gallic acid	$C_7H_6O_5$	170	0.5	0.5			
Quinic acid	$C_7H_{12}O_6$	192	2.0	2.0			
Chlorogenic acid	$C_{16}H_{18}O_9$	354	Trace	Trace			
p-Coumarylquinic	$C_{16}H_{18}O_8$	338	Trace	Trace			
Theaflavins (TFs)				3–6	Mouth drying, rough astringent		Yellowish brown
Theaflavin (TF)	$C_{29}H_{24}O_{12}$	564		Trace			
Isotheflavin	$C_{29}H_{24}O_{12}$	564		Trace			
Neotheaflavin	$C_{29}H_{24}O_{12}$	564		Trace			
Theaflavin-3-monogallate (TF1)	$C_{36}H_{28}O_{16}$	716		Trace			
Theaflavin-3′-monogallate (TF2)	$C_{36}H_{28}O_{16}$	716		Trace			
Theaflavin-3,3′-gallate (TF3)	$C_{43}H_{32}O_{20}$	868		Trace			
Theaflavic acid	$C_{21}H_{16}O_{10}$	428		Trace			
Epitheaflavic acid	$C_{21}H_{16}O_{10}$	428		Trace			
Epitheaflavic acid gallate	$C_{28}H_{20}O_{14}$	580		Trace			
Thearubigins (TRs)				12–18	Ashy and slight astringent		Reddish brown
Thearubigin-1 (TR1)				Trace			
Thearubigin-2 (TR2)				Trace			
Thearubigin-3 (TR3)				Trace			
Others							
Bisflavanol A	$C_{44}H_{34}O_{22}$	914		Small amount		Astringent and bitter	
Bisflavanol B	$C_{37}H_{30}O_{18}$	762		Trace			
Bisflavanol C	$C_{30}H_{26}O_{14}$	610		Trace			
Tricetinidin	$C_{15}H_{11}O_6$	287		Trace			
Purpurogallin-6-carboxylic acid	$C_{12}H_8O_7$	264		Trace			
Categallin	$C_{20}H_{16}O_8$	384		Trace			
Pyrogallin	$C_{20}H_{16}O_9$	400		Trace			
Carotenoids							Yellow
β-carotene	$C_{40}H_{56}$	537	0.006	0.006			
Lutein	$C_{40}H_{56}O_2$	569	0.008	0.007			
Violaxanthin	$C_{40}H_{56}O_4$	601	0.002	0.001			
Neoxanthin	$C_{40}H_{56}O_4$	601	0.003	0.003			
Theanine	$C_7H_{14}N_2O_3$	174	3	3	Brothy		
Caffeine	$C_8H_{10}N_4O_2$	194	3–6	3–6	Bitter and creamy		
Pectin			3	3			
Proteins			6–15	5–15			
Amino acids			6	6	Brothy		
Ash			5	5			
Cellulose	$(C_6H_{10}O_5)_n$		5–7	5–7			
Carbohydrates			10–15	10–15			
Lignin			6.0	4–6			
Lipids/organic acid			2–8	2–8			
Volatile compounds			0.01	0.01			

appearance to make tea black. It is notable that the lower the amount of TRs, the poorer the creaming characteristic of brew (Drynan et al., 2010a). The formation of TRs begins at plucking and continues up to the drying stage of the black tea processing. During the rolling and fermentation stages of black tea manufacture, TRs increase steadily, while catechins, gallocatechins,

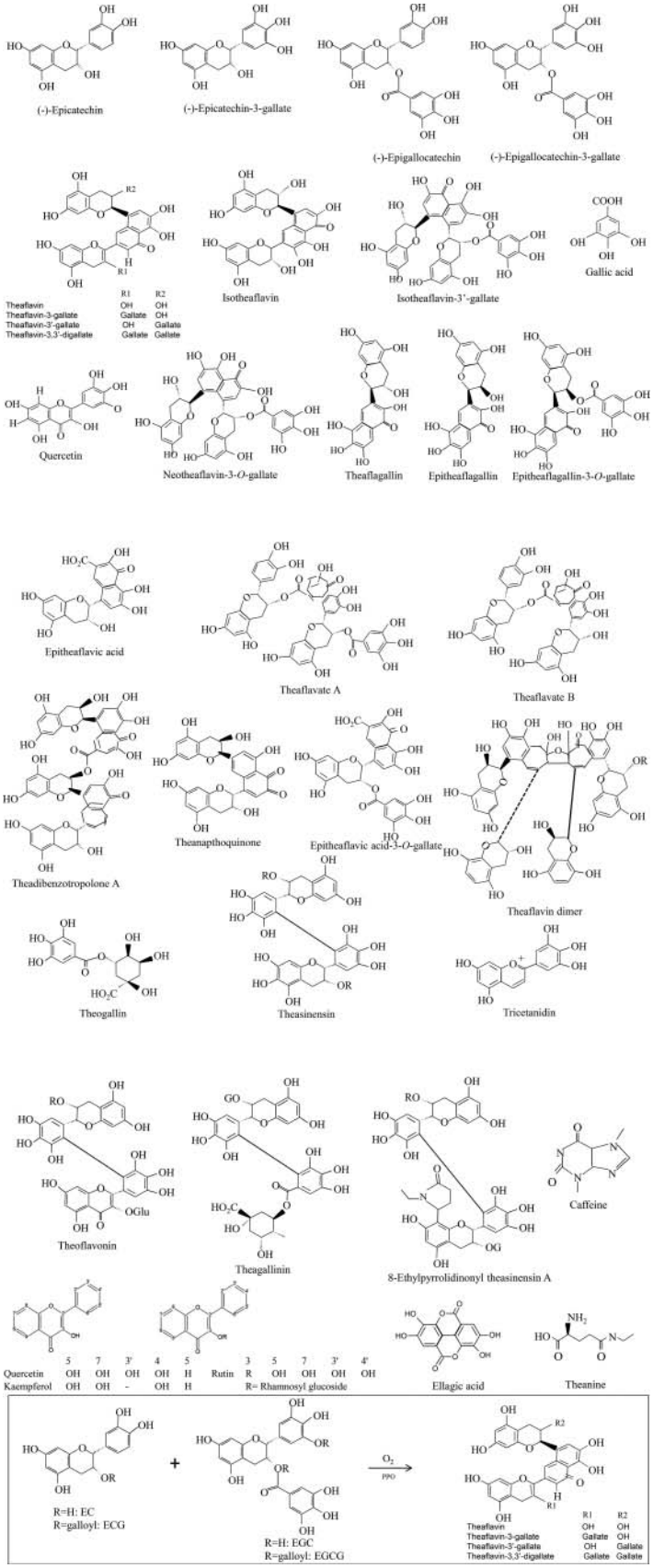

Figure 1. Chemical structure of tea bioactive phytochemicals and formation of TFs from catechins.

and their gallates along with flavonol glycosides decrease throughout this period. It has been also observed that during rolling the leaves, the PPO activity predominates, and favoring TFs are formed, and with the progress of oxidation and fall of pH, PO activity supports the generation of TRs. In water deficient conditions (withered leaves), the concentration of PO is

considerable, facilitating the formation of TRs, but in a water-rich reaction medium, its action is hindered, but the PPO activity enhances, resulting in more formation of TFs. Table 1 shows the list of phenolics and color formed during the rolling oxidation stage of black tea. Different ratios of TFs and TRs contribute to different shades of black tea (Mahanta, 1988).

Moreover, the yellow color of brew is mainly due to the presence of water-soluble flavonols (kaempferol, quercetine, isoquercetin, myricetin, myricitrin, rutin, and kaempferitrin), flavones (apigenin, isovitexin, vitexin, saponarin, and vicenin-2), and their glycosides. Of these, quercetin-3-O-(6-O-α-l-rhamnopyranosyl)-β-D-glucopyranoside (rutin) has a very low taste threshold level and is therefore important in determining the tea taste (Park and Dong, 2003; Drynan et al., 2010b; Butt et al., 2014).

Amino acids

Black tea contains numerous amino acids, but L-theanine (γ-glutamylethylamide) is a unique amino acid accounting for 1–2% of dry weight, which makes 50% of the total amino acids (Table 1). It appears to occur in only one mushroom species and two other species of the *Camellia* genus. The degradation of theanine is greatly involved in the biogenesis of aroma and taste of tea (Mahanta, 1988). It is synthesized in the roots, and transports to the leaves, where sunlight converts theanine into polyphenols. Because of this, some tea cultivators grow their plants out of direct sunlight to preserve theanine content, and thus the flavor. Some amino acids, such as arginine and alanine, contribute the bitterness of tea infusion. Moreover, lysine, glutamine, aspartic acid, threonine, glutamic acid, glycine, α-alanine, arginine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, asparagine, and tryptophan are also detected in black tea. During processing, some of these amino acids are transformed into volatile aroma compounds (Sharma and Rao, 2009). For example, valine, methionine, isoleucine, leucine, and phenylalanine transform into isobutyraldehyde, methional, 2-methyl butanal, isovaleraldehyde, and phenyl acetaldehyde, respectively. During the heating process, amino acids interact with polyphenols to yield colored compounds, while amadori is formed when amino acids interact with glucose, which improves the flavor of brew (Li et al., 2013).

Volatile flavor compounds

The popularity of tea as a universal beverage depends upon its soothing flavor together with its stimulating effects. The principle bioactive constituents, which influence the flavor, include volatile flavor compounds (VFCs). These volatiles become recognizable only when tea leaves have withered, rolled, oxidized, and fired (Li et al., 2013). Volatile fractions of tea leaves have been studied in detail, and more than 600 different compounds have been identified (Sang et al., 2011). Major VFCs characterized in black tea are linalool, benzyl alcohol, nonanal, phenyl acetaldehyde, n-hexanal, methyl salicylate, E-2-hexenal, Z-3-hexenol, 2-hexenol, n-hexanol, n-heptanal, benzaldehyde, (E,Z)-2,4-heptadienal, (E,E)-2,4-heptadienal, cis-linalool oxide, trans-linalool oxide, phenyl ethyl alcohol, α-terpineol, cis-geraniol, indole, geranyl acetate, β-ionone, dihydroactinidiolide, nerolidol, and phytol (Table 2 and Fig. 2). Among these, linalool is the main essence in black tea. Moreover, the degraded products of amino acids, carotenoids, and linoleic acid are also responsible for brew's flavor. Primeverosides, such as 4-O-β-D-glucopyranosides and 6-O-dxylopyranosyl-O-D-gluco-pyranosides, act as key aroma precursors developed during the rolling of tea leaves. In addition, vicianosides, acuminosides, aglycons of (Z)-3-hexenol, geraniol, linalool, linalool oxides, benzyl alcohol, 2-phenyl ethanol, and methyl salicylate are other primeverosides, bio-transformed into their VFCs (Li et al., 2013).

Methyl xanthines

Methyl xanthines are present as caffeine (1, 3, 7-trimethyl xanthine) and small amounts of theophylline and theobromine. Caffeine is a derivative of purine and the richest alkaloid among xanthine molecules, constituting 3–4% of the dry weight of tea leaves (Table 1). It is the most stable molecule and remains unchanged during all the processes that it undergoes. Synergistic effects of caffeine and polyphenols are required for the development of reasonable amounts of tangy astringency and creamy property of brew. According to Ulrich et al. (2000), decaffeination may change the nature of astringency from tangy to non-tangy-type. The content of caffeine in a cup of tea beverage depends on several factors such as variety, processing method, brewing time, amount of tea powder, and the quantity of water used (Feng et al., 2002), although the amount of caffeine is higher in tea leaves than roasted coffee beans. Theobromine has been associated to enhance the rate of metabolism, while theophylline has been reported to be a bronchodilator in pure form. The amounts of theophylline and theobromine vary dramatically in tea products to have possible health promoting effects (Sharma and Rao, 2009).

Miscellaneous constituents

The main shade of color in the infused leaf of black tea can be quantified by measuring the ratio of chlorophyll a (dark green) and chlorophyll b (yellowish-green). The chlorophyllase-dependent bio-transformation of chlorophyll into pheophytin and pheophorbide under high temperature and humidity may cause the tea color to become darker. Carotenoids, lipids, and anthocyanins are not only the major constituents present in a black tea brew but they also contribute the therapeutic effects to tea and are responsible for the development of aroma and color (Table 1). Carbohydrates, such as glucose, fructose, sucrose, raffinose, stachyose, maltose (in Assam tea), and rhamnose (in China tea), are associated to produce heterocyclic flavor compounds (Mahanta, 1988). Several minerals, such as fluorine, potassium, aluminum, iodine, selenium, nickel, and manganese, comprise about 4 to 9% of inorganic matter of tea. Other chemical compounds such as phytoestrogens and vitamin C are also detected in black tea (Li et al., 2013).

Table 2. Volatile compounds responsible for aroma and flavor of tea.

Compound	Molecular formula	Molecular weight	Flavor	Found in	Characteristic MS data (m/e)
Alkanals/alkenals					
3-Methyl butanal	$C_5H_{10}O$	86	Sweet, stimulus	B, F	57, 29, 41, 58
Z-3-pentenal	$C_5H_{10}O$	84	Green notes	B	55, 29, 27, 41
2-Methyl pentanal	$C_6H_{12}O$	100		B	43, 58, 41, 71
3-Methyl pentanal	$C_6H_{12}O$	100		DB	56, 41, 43, 44
Hexanal	$C_6H_{12}O$	100	Green notes	B	44, 56, 41, 43
E-2-Haxenal	$C_6H_{10}O$	98	Green notes	B, G, F, P	41, 55, 69, 83
Z-3-Hexenal	$C_6H_{10}O$	98	Leafy odour	F	41, 55, 69, 83
E-2-Z-4-Hexadienal	C_6H_8O	96	Green notes	B	81, 39, 41, 96
2,4-Dimethyl-2,4-hetadienal	$C_9H_{14}O$	138		B	109, 41, 67, 138
E-2-Z-4-Heptadienal	$C_7H_{10}O$	110	Green notes	B	81, 39, 53, 67
E-2-Z-4-Octadienal	$C_8H_{12}O$	124	Green notes	B	81, 39, 41, 67
E-2-Z-4-Non-adienal	$C_9H_{14}O$	138	Fresh woody note	B	67, 81, 95, 109
E-2-Z-4-Decadienal	$C_{10}H_{16}O$ $C_5H_{10}O$	152	Fresh woody note	B	67, 81, 95, 109
Alkanones/alkenones					
3-hydroxy-2-butanone	$C_4H_8O_2$	88		B	45, 43, 88, 42
2,3,-butadione	$C_4H_6O_2$	86	Buttery, nutty	B	43, 15, 86, 42
1-Penten-3-one	C_5H_8O	84		B	55, 84, 56, 57
3-hydroxy-pentan-2-one	$C_5H_{10}O$	86		B	59, 43, 41, 58
2-Heptanone	$C_7H_{14}O$	114		B	43, 58, 71, 59
2-Methyl-2-hepten-6-one	$C_8H_{14}O$	126		B, P	43, 41, 69, 55
E-3-E-5-Octadien-2-one	$C_8H_{12}O$	124		B, G, F	43, 95, 81, 53
2-Octanone	$C_8H_{16}O$	128		B	43, 58, 59, 71
Alkanols/Alkenol					
Pentan-2-ol	$C_5H_{12}O$	88	Green notes	B	45, 41, 43, 73
1-Penten-3-ol	$C_5H_{10}O$	86	Green notes	B, G, F, P	57, 29, 27, 31
Hexanol	$C_6H_{14}O$	102	Hay-like notes	B, G, F	56, 43, 55, 42
1-Hexen-3-ol	$C_6H_{12}O$	100		B	57, 43, 72, 41
Z-3-Hexen-1-ol	$C_6H_{12}O$	100	Green sweet notes	B, G, F, P	41, 67, 55, 82
E-2-Hexen-1-ol	$C_6H_{12}O$	100	Green notes	B, G	31, 41, 57, 67
2-Heptanol	$C_7H_{16}O$	116		DB	45, 43, 55, 41
1-Octen-3-ol	$C_8H_{16}O$	128		B	57, 72, 85, 81
1,5-Octadien-3-ol	$C_8H_{14}O$	126		DB	57, 70, 55, 41
1-Octanol	$C_8H_{18}O$	130		B, G, F	31, 42, 56, 70
1-Nonanol	$C_9H_{20}O$	144		DB	69, 41, 87, 43
Acids					
Butanoic	$C_4H_8O_2$	88	Fatty notes	B, G, F, P	60, 73, 27, 41
2-Methyl butanoic	$C_5H_{10}O_2$	102	Fatty notes	B	74, 57, 29, 41
Pentanoic	$C_5H_{10}O_2$	102	Fatty notes	B, G, F	60, 73, 41, 43
Hexanoic	$C_6H_{12}O_2$	116	Fatty notes	B, G, F, P	60, 73, 41, 27
Z-3-Hexenoic	$C_6H_{10}O_2$	114	Fatty notes	B, G, F	41, 39, 68, 73
Benzoic	$C_7H_6O_2$	122		B, G	100, 122, 78, 51
Phenyl acetic	$C_8H_8O_2$	136	Floral	B	91, 136, 92, 65
Geranoic	$C_{10}H_{16}O_2$	168	Green floral	B	69, 41, 39, 100
Esters					
1-Hydroxy-2-propan acetate	$C_5H_8O_4$	132		DB	43, 87, 42, 44
Butyl acetate	$C_6H_{12}O_2$	116		B	43, 94, 58, 82
E-2-Hexenyl formate	$C_7H_{12}O_2$	128		B	67, 41, 57, 82
Methyl hexanoate	$C_7H_{14}O_2$	130		B, F	74, 87, 59, 99
E-3-Hexenyl acetate	$C_8H_{14}O_2$	142		B, F	43, 67, 82, 41
Phenyl acetate	$C_8H_8O_2$	136		DB	43, 94, 58, 42
Ethyl benzoate	$C_9H_{10}O_2$	150		DB	105, 77, 51, 122
Benzyl benzoate	$C_{13}H_{10}O_2$	198		DB	105, 91, 77, 51
Methyl tetradecanoate	$C_{15}H_{30}O_2$	242		DB	74, 87, 43, 41
Methyl heptadecanoate	$C_{18}H_{36}O_2$	284		DB	74, 87, 43, 55
Methyl palmitate	$C_{17}H_{34}O_2$	270		G	74, 43, 87, 41
Ethyl palmitate	$C_{18}H_{36}O_2$	284		G	88, 101, 43, 41
Lactones					
2-Methyl-4-butanolide	$C_5H_8O_2$	100	Nut-like	DB	41, 56, 42, 100
Methyl-4-butanolide	$C_8H_{14}O_2$	142		B	85, 41, 55, 42
5-Octanolide	$C_8H_{14}O_2$	142	Sweet creamy	B	42, 99, 71, 55
5-Nonanolide	$C_9H_{16}O_2$	156	Nut-like odour	DB	42, 99, 41, 43
5-Decanolide	$C_{10}H_{18}O_2$	170	Typical musky	B	85, 128, 29, 41
Coumarin	$C_9H_6O_2$	146		B, G, P	146, 117, 90, 89
Dihydroactinidiolide	$C_{11}H_{16}O_2$	180	Peach-like	B, G, F, P	111, 43, 67, 41
Cis-Jasmone (7) (3-methyl-2- (Pent-2-en) cyclopent-2-en-1-one)	$C_{11}H16O$	164		B, G, F, P	164, 110, 149, 79
Furfural	$C_5H_4O_2$	96	Caramel, nutty	B, G	39, 96, 95, 67
2-Ethylfuran	$C_6H_8O_2$	96		B	81, 96, 53, 39
2,5-Furandione	$C_4H_2O_3$	98		B	54, 53, 98, 41
2-Pentylfuran	$C_9H_{14}O$	138	Beany grassy	B	81, 53, 138, 39

(*Continued on next page*)

Table 2. (Continued).

Compound	Molecular formula	Molecular weight	Flavor	Found in	Characteristic MS data (m/e)
2,6,6-Trimethyl-2-vinyl tetrahydropyran	$C_{10}H_{18}O$	154		DB	68, 67, 110, 43
Toluene	C_7H_8	92	Solvent-like	B	92, 93, 91, 66
O-xylene	C_8H_{10}	106	Caramel, toffee-like	B	91, 106, 105, 65
Benzaldehyde	C_7H_6O	106	Fruity	B, G, F, P	77, 105, 106, 43
Phenyl acetaldehyde	C_8H_8O	120	Floral	B, P	91, 120, 92, 65
Guaicol (O-methoxyphenol)	$C_7H_8O_2$	124	Smoky note	B	109, 119, 81, 42
Thymol	$C_{10}H_{14}O$	150		B	135, 150, 91, 117
Methyl salicylate	$C_8H_8O_3$	152	Winter green	B, G, F	39, 65, 92, 120
Naphthalene	$C_{10}H_8$	128		G	128, 129, 127, 51
2-Methylnaphthalene	$C_{11}H_{10}$	142		DB	156, 141, 155, 157
1,7-Dimethylnaphalene	$C_{12}H_{12}$	156		DB	156, 141, 155, 157
2-Furfuryl methyl ketone	$C_7H_6O_2$	122		DB	43, 81, 82, 53
1-Indanone	C_9H_8O	132		DB	132, 104, 78, 42
Benzyl alcohol	C_7H_8O	108		B, G, F, P	79, 108, 107, 77
2-Phenyl ethanol	$C_8H_{10}O$	122		B, G, F, P	91, 92, 122, 65
2-Butyl furan	$C_8H_{12}O$	124		DB	91, 124, 82, 53
Terpenoids/Hydrocarbons					
Limonene	$C_{10}H_{16}$	136	Sweet and floral	B, G, F	68, 79, 93, 107
E-β-Ocimene	$C_{10}H_{16}$	136	Pleasant	B, G	93, 80, 79, 77
Camphene	$C_{10}H_{18}$	138		DB	93, 41, 79, 121
β-Myrecene	$C_{10}H_{16}$	136	Pleasant	B	41, 69, 93, 79
Δ-3-Carene	$C_{10}H_{16}$	136		DB	93, 41, 91, 77
p-Cymene	$C_{10}H_{14}$	134		B	119, 91, 134, 117
α-Cubene	$C_{15}H_{24}$	204		G	161, 119, 105, 81
α-Cadinene	$C_{15}H_{24}$	204		G	161, 119, 105, 93
β-Elemene	$C_{15}H_{24}$	204		B	121, 93, 41, 79
L-Humulene	$C_{15}H_{24}$	204	Slightly pappery	B, G	93, 41, 69, 80
Caryophyllene	$C_{15}H_{24}$		Woody, Spicy	G, P	41, 69, 93, 133
Alcohols					
Linaool	$C_{10}H_{18}O$	154	Sweet	B, G, F, P	71, 93, 41, 43
E-Furan-linalool oxide	$C1_5H_{18}O2$	170	Sweet	B, G, F	59, 43, 94, 111
Z-Furan-linalool oxide	$C_{10}H_{18}O2$	170	Sweet	B, G, F	59, 43, 94, 121
E-Pyran-linalool oxide	$C_{10}H_{18}O2$	170	Sweet	B, G, F, P	110, 95, 152, 67
Z-Pyran-linalool oxide	$C_{10}H_{18}O2$	170	Sweet	B, G, F, P	110, 95, 152, 67
Myrtenol	$C_{10}H_{18}O$	154		DB	79, 91, 41, 43
Nerol	$C10H_{18}O$	154	Rosy	B, G, P	69, 41, 39, 93
Fenchyl alcohol	$C_{10}H_{18}O$	154		B	81, 80, 69, 82
Geraniol	$C_{10}H_{18}O$	154	Rosy notes	B, G, F, P	69, 41, 39, 64
Carveol	$C_{10}H_{16}O$	152	Spicy note	DB	119, 91, 13, 92
α-Terpineol	$C_{10}H_{18}O$	154	Fine, delicate odour	B, G, F, P	59, 93, 43, 81
Citronellol	$C_{10}H_{20}$	156	Rosy	B	41, 69, 55, 67
Nerolidol	$C_{15}H_{26}O$	222	Woody floral	B, G, F, P	69, 41, 93, 43
Cedrol	$C_{15}H_{26}O$	222		B, G	95, 150, 81, 43
β-Eudesmol	$C_{15}H_{26}O$	222	Sweet woody	DB, G	59, 109, 149, 189
Phytol	$C_{20}H_{40}O$	296		B	71, 43, 57, 41
Ketones					
2,6,6-Trimethyl cyclohex-2-en-1-one	$C_9H_{14}O$	130		B	82, 54, 138, 39
2,6,6-Trimethyl cyclohexanone	$C_9H_{16}O$	140		B	82, 56, 69, 55
2,6,6-Trimethyl cyclohex-2-en, 1,4-dione	$C_9H_{12}O2$	152		B, G	68, 96, 40, 152
2,6,6-Trimethyl-4-hydroxycyclo hex-1-one	$C_9H_{16}O2$	156		B, G	83, 57, 41, 69
5-(Methylethyl)-E-3-hepten-2-one	$C_6H_{12}O$	154		DB	43, 97, 112, 55
3,3-Dimethyl-2,7-octadione	$C_{10}H_{18}O$	168		B	43, 69, 109, 86
Fenchone	$C_{10}H_{16}O$	152	Harsh fennel odour	B	81, 68, 51, 152
Damascenone	$C_{13}H_{18}O$	190	Floral	B	69, 121, 41, 105
α-Damascenone	$C_{13}H_{20}O$	192	Floral	B	69, 123, 81, 41
β-Damascenone	$C_{13}H_{20}O$	192	Floral	B	177, 69, 41, 121
1,5,5,9-Tetramethyl bicycle(4–3–0)non-8en-7-one	$C_{13}H_{20}O$	192	Floral	B	110, 123, 177, 41
α-Ionone	$C_{13}H_{20}O$	192	Floral	B, G, P	43, 121, 93, 136
β-Ionone	$C_{13}H_{20}O$	192	Warm woody	B, G	177, 43, 41, 93
Geranyl acetone	$C_{13}H_{22}O$	194		B	43, 69, 93, 125
4-Oxo-β-ionone	$C_{13}H_{18}O2$	206		B	43, 163, 121, 206
5,6-Epoxy-β-ionone	$C_{13}H_{20}O2$	208		B, P	123, 43, 135, 109
Dehydro vomifoliol-(1′-hydroxy 4′-oxo-α-ionone)	$C_{13}H_{20}O2$	208		B	124, 43, 166, 95
Z-Theaspirane	$C_{13}H_{22}O$	194		B	138, 82, 96, 83
Z-Dihydrotheaspirene	$C_{13}H_{22}O2$	210		B	43, 126, 154, 55
Z-6-Hydroxy dihydrotheaspirane	$C_{13}H_{24}O2$	212		B	85, 43, 126, 86
β-Cyclocitral	$C_{10}H_{16}O$	152	Warm woody	B	41, 81, 109, 123
Geranyl formate	$C_{11}H_{18}O2$	182	Green rosy	B	69, 41, 39, 68
Methyl-E-dihydro jasmonate	$C_{13}H_{22}O3$	224	Sweet floral	B, G	83, 156, 153, 82
2,5-Dimethyl pyrrole	C_6H_8O	94		B	94, 95, 42, 80
2-Formyl purrole	C_6H_5NO	95	Smoky note	B	109, 108, 53, 80
Pyridene	C_5H_5N	79		B	79, 52, 51, 50

(*Continued on next page*)

Table 2. (Continued).

Compound	Molecular formula	Molecular weight	Flavor	Found in	Characteristic MS data (m/e)
2-Ethyl pyridine	C_7H_9N	107		B	104, 105, 79, 80
Methyl pyridine	C_5H_6N2	94	Pungent odour	R	94, 67, 39, 40
2,6-Dimethyl pyrazine	C_6H_8N2	108	Pungent odour	B, R	43, 108, 39, 40
2-Acetyl pyrazine	C_6H_6N2O	122		B	43, 52, 53, 94
Indole	C_8H_7N	117		B, G, F, P	117, 90, 91, 63
3-Methyl indole	C_9H_9N	131		DB, G	130, 131, 43, 42
Dimethyl sulphide	C_2H_6S	62		B, G	47, 62, 45, 46

B: Black tea; G: Green tea; DB: Darjeeling black tea; F: Fresh leaves tea; P: Pouchong tea; R: Roasted green tea.

Chemopreventive properties

Antioxidant activity

Overproduction of FRs causes oxidative damage to biomolecules, resulting in the development of numerous diseases, including cancer, cardiovascular, atherosclerosis, inflammatory injury, aging, and neurodegenerative diseases (Singh et al., 2009a, 2009b, 2009c, 2011a). In recent years, increasing attention has been paid to the role of diet in human health. Several epidemiological studies have indicated that a high intake of fruits, vegetables, spices, and medicinal plants is associated with a reduced risk of cancer (Aggarwal and Shishodia, 2004, 2006; Aggarwal et al., 2008; Singh et al., 2011b; Shukla et al., 2014; Singh et al., 2014). Antioxidants such as flavonoids and phenolic acids are found in various plants (Singh et al., 2009b). They have been found to reduce the access of the most damaging FRs due to their ability to scavenge oxygen–nitrogen-derived FRs by donating hydrogen atom or an electron, chelating metal catalysts, activating antioxidant enzymes, and inhibiting oxidases (Prakash et al., 2007; Singh et al., 2009c). Based on accumulative evidence, in recent decades, tremendous interest has considerably increased in finding natural antioxidants present to replace synthetic antioxidants, which are being restricted due to their side effects. On the other hand, flavonoids, used as natural antioxidants, are gaining importance due to their range of

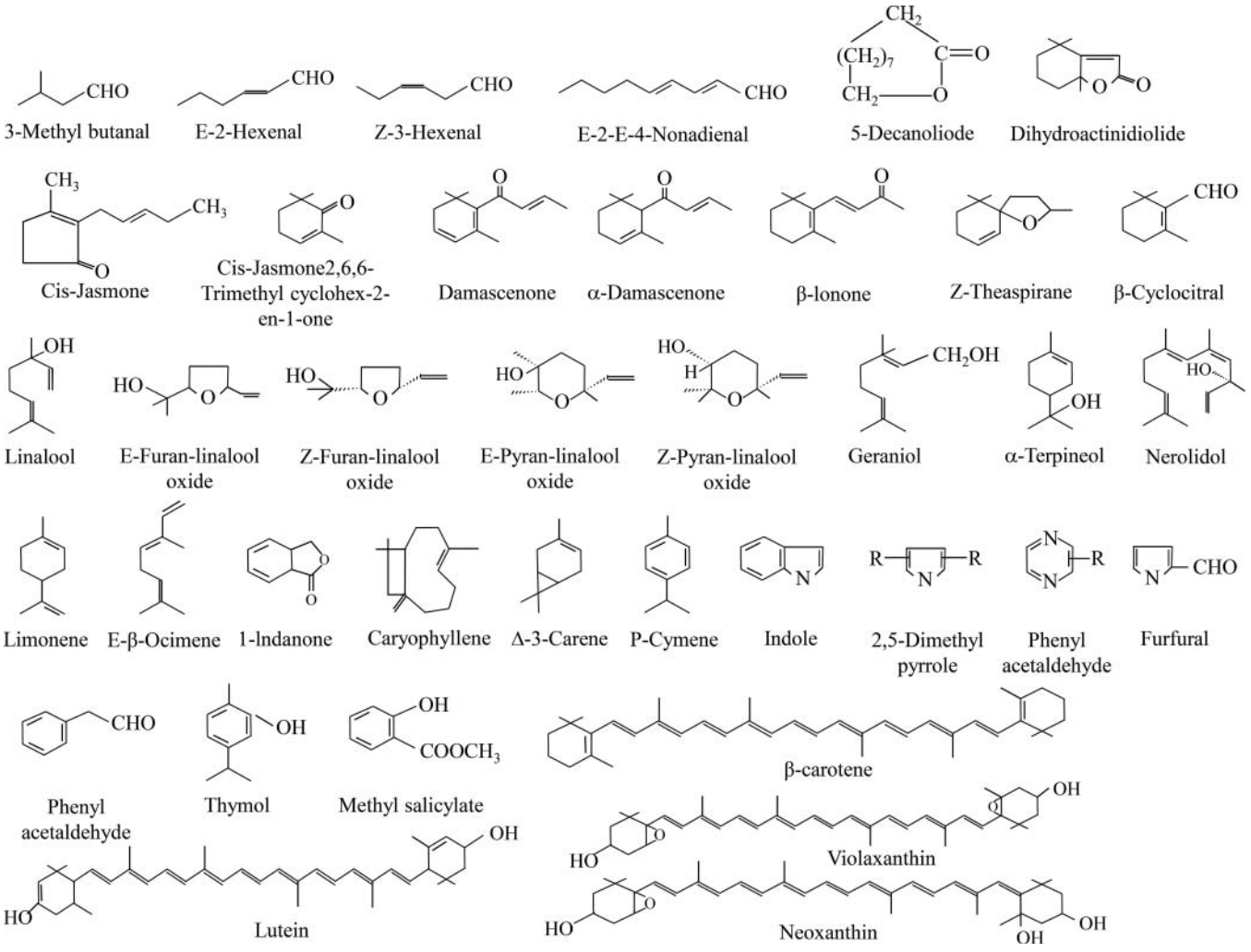

Figure 2. Flavor-related volatile constituents of tea.

biological activities, decreasing the risk of various cancers (Katiyar and Mukhtar, 1997b; Singh et al., 2009c).

Certain bioactive constituents present in black tea possess antiradical activity. Thus, tea drinking elicits the endogenous antioxidant gene pool and reduces the risk of various types of cancers (Katiyar and Mukhtar, 1997b). Black tea contributes 60–84% of dietary flavonoids in Western populations, and it has been reported that consumption of flavonoids by tea drinkers is 20 times more than by non-consumers of tea (Song and Chun, 2008). Polyphenols such as TFs, TRs, and gallic acid present in black tea are reported to be responsible for its strong antioxidant activity. A plethora of evidence suggests that the black tea polyphenols can protect cells from FR-mediated oxidative damage (Fig. 3). Luo and co-workers (2012) observed that oxidative stress, inflammation, and hepatocyte apoptosis increased in steatotic liver compared with a normal liver. However, administration of TF1 was found to significantly decrease the levels of these changes. In LPS-stimulated RAW264.7 cells, the decreased production of reactive oxygen species (ROS) and TNF-α by TF1 is also examined.

Numerous studies have shown that black tea consumption has equal antioxidant and FR scavenging effects as to those drinking green tea. Black tea TFs and TRs possess at least the same antioxidant potential as catechins present in green tea (Feng et al., 2002; Erba et al., 2003; Singh et al., 2011b). Oxidative damage to lipoproteins is related to atherogenesis. Flavonoids present in black tea inhibited the oxidation of lipids, thereby preventing atherosclerosis (Sharma and Rao, 2009). Pretreatment of black tea was found to inhibit the frequency of chloropyriphos and cypermethrin-induced LPO in mice liver. This protective effect was associated with the upregulation of endogenous antioxidants, including SOD, CAT, GR, GPx, GST, GSH, and total thiol. These enzymatic proteins are known to efficiently quench oxidative burden by minimizing damage to cells. This study suggests that black tea can help in the revival of endogenous antioxidant system. Among the polyphenols of black tea, TFs are the main activity-modulating components (Katiyar and Mukhtar, 1997b; Feng et al., 2002).

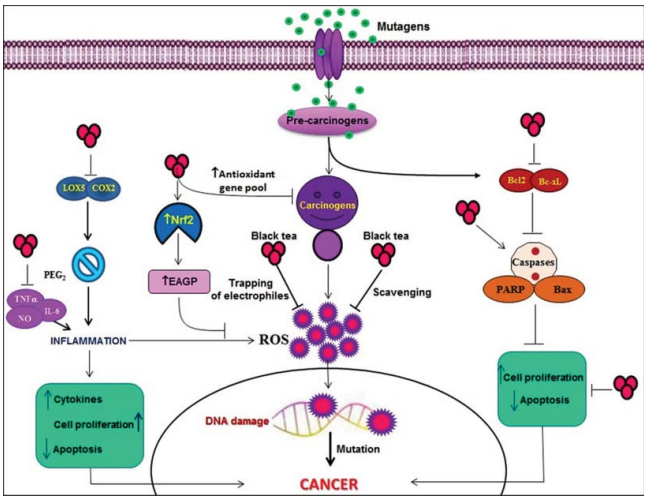

Figure 3. Mechanistic representation of antioxidant, anti-mutagenic, and chemopreventive properties of black tea. Several environmental factors, including UV light, ozone, tobacco smoke, different xenobiotics, ionizing radiation herbicides, and pesticides, generate ROS in excess amount. These ROS are generated during the bio-transformation of mutagens into pre-carcinogens, thereby inducing DNA damage, protein aggregation, and lipid peroxidation. COX-2, 5-LOX, TNFα, IL-6, and excessive and prolonged NO generation are linked with inflammation and tumorigenesis. Apoptosis is a well-orchestrated process controlled by multiple pro-apoptotic and anti-apoptotic genes, particularly the Bcl-2 gene family. Antioxidant polyphenols of black tea prevent cancer by scavenging ROS, inhibiting inflammation-inducing enzymes, and by controlling cell proliferation through induction of apoptotic protein expression (Bax, PARP, and caspases). The various protective mechanisms are marked with a blunt headline.

Studies have cleared that a group of TFs in black tea, specifically TF-3 and TF-4, showed strong antioxidant activity, which was similar to EGCG of green tea. Therefore, these TFs inhibit inflammation, clastogenesis, and several types of cancers (Sharma and Rao, 2009). Interestingly, EGCG alone was found to considerably affect LPO in jarkut cells; however, along with black tea extract, no protective effect was observed. Authors concluded that some components of black tea might be responsible for loss of antioxidant effect of EGCG. At 5 μg/mL, black tea extract showed radioprotection in normal lymphocytes. TFs blocked LPO in erythrocyte membrane ghosts and microsomes. This antioxidant effect was related to FR scavenging of galloyl moiety of TFs. Alcohol intake causes oxidative stress by generating FRs in the cells, resulting in the depletion of antioxidant system. TFs were found to significantly protect against alcohol-induced oxidative damage and partial protection to the brain cells. The maximum antioxidant level was examined in the liver of mice (Luczaj and Skrzydlewska, 2004).

The antioxidant potential of hot water extract of black tea was studied by evaluating radioprotection conferred to pBR322 DNA, calf thymus DNA, and normal lymphocytes during gamma irradiation (Fig. 3). The highest protection was observed in pBR322 DNA followed by calf thymus with IC_{50} with 182 μg/mL. Oxidative DNA damage generates 8-hydroxy-2′-deoxyguanosine, which is used as a plasma or urinary marker for exposure to chemical carcinogens or radiation (Ghosh et al., 2012). TFs were recorded to decrease DNA damage and mutagenesis by enhancing antioxidant function and suppressing cytochrome P450 1A1 in the normal liver epithelium RL-34 cells of rat. Protective effect of polyphenols was found to be in the following order: TF4 > TF3 > TF2 (Feng et al., 2002). Serafini and colleagues tried to compare antioxidant activity of phenol-rich beverages such as black tea, green tea, red wine, and white wine. The antioxidant capacity of red wine was found to be higher than in the tea using low-density lipoprotein (LDL) oxidation assay, an in vitro system. The phenolic content and the antioxidant activity were reported in the following order: red wine > green tea > black tea > white wine. Unexpectedly, under the in vivo model system, black tea showed higher antioxidant activity than green tea and red wine. This occurred due to changes in the structure of polyphenols during digestion and assimilation. A number of human studies supported this interpretation. However, work on animals showed that the black tea extract improves plasma lipid profiles and reduces the oxidation of LDL and very low density lipoprotein (VLDL), followed by a high cholesterol diet (Sharma and Rao, 2009). Inhibitory effect of black tea on LDL oxidation and fatty acid synthase suggests a key role in the prevention of cardiovascular diseases. Black tea consumption provided protection against nitrous oxide and superoxide ($O_2^{\bullet -}$) in murine peritoneal macrophages (Sharma and Rao, 2009). Many researchers also documented that TFs are the most effective compounds in

down-regulating oxidative stress and found a better chemo-preventive agent than green tea.

Pre-Treatment with black tea extract inhibited carbon tetrachloride-induced LPO by 49% and 37% in the liver of female and male rats, respectively. Similar protective effect was also observed in the kidneys and testes of rats. This effect was related with the scavenging of FRs induced by carbon tetrachloride (Fadhel and Amran, 2002). Flavonoid-rich black tea extract also inhibited blue sprat-mediated oxidation of linoleic acid. Its aqueous extract was also found to prevent cross-linking of proteins, and inhibited oxidative DNA strands breakage. Moreover, the reduced oxidative stress induced by cigarette smoking was also observed in the black tea extract-treated animals (Sharma and Rao, 2009). Within first 5 min of brewing, approximately 84% antioxidant activity was solubilized, while an additional 13% activity was extracted when tea was brewed for another 5 min.

In a randomized study, Henning et al. (2004) approached 30 healthy subjects for consuming a single bolus of black or green tea, and observed maximum antioxidant status of plasma at 2 h after their consumption. It was analyzed by Oxygen Radical Absorbance Capacity assay (Prior and Cao, 1999) that black tea had a mean antioxidant capacity of 761.1 μmol Trolox equivalent to per gram dry matter. Rietveld and Wiseman (2003) examined the powerful antioxidant activity of black tea flavonoids in in vitro; however, no activity was found in in vivo systems. Improvement in human vascular function was observed when drinking flavonoid-rich black tea (450 mL/day) for four days (Widlansky et al., 2005). This study was supported by a study conducted by Davies et al. (2003), in which improvement in coronary heart disease (CHD) risk factors was examined, but no noticeable changes in antioxidant status were determined. A considerable body of observational evidence revealed that the intake of black tea and its flavonoids improve heart health and regulate the process of carcinogenesis, diabetes, inflammation, thrombosis, and endothelial function by enhancing endogenous antioxidant system (Sharma and Rao, 2009).

Anti-mutagenic activity

Both intracellular and extracellular mechanisms seem to be involved in the anti-mutagenic activity of black tea (Table 3). These include modulation of metabolism, DNA replication and repair effects, and promotion of invasion and metastasis (Fig. 3). Not only green tea flavonoids but black tea polyphenols have also been reported to bring benefits in lowering oxidative stress and exerting anti-mutagenic effect (Sharma and Rao, 2009). TFs cause dose-dependent inhibition of Cd-induced DNA damage in rat testis and enhance the levels of serum testosterone and sperm characteristics. TFs also reduce LPO and Cd concentration in urine, feces, liver, testis, and blood (Wang et al., 2012b). TFs also suppress H_2O_2-induced mutagenicity in *Salmonella typhimurium* TA104. Exposure of *Escherichia coli* to black tea extract was observed to inhibit the mutagenicity of *N*-Methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) because it contained low molecular weight tannins, including gallic acid (Roy et al., 2001).

Table 3. Anti-mutagenic activity of black tea.

Mutagen	Responsible components	Bio-activity	Mode of action	Experimental model	Reference
B(a)P	TFs	Antimutagenic	Inhibition of CyP450-1A1 Prevention of DNA damage	Hep G2 cells	Feng et al., 2002
Cyclophosphamide and DMBA	TFs and TRs	Anticlastogenic		Mouse bone marrow cells	Gupta et al., 2001
Mitomycin	Green, black tea and tannin	Antimutagenic		Mouse bone marrow cells	Imanishi et al., 1991
Hydrogen peroxide	TF2, T3, and TF4	Antimutagenic			Mazumdar et al., 2013
Mutant H ras gene	Black and green tea polyphenols	Antimutagenic	Inhibition of AP-1 activity. Inhibition of cell proliferation	Epidermal JB-6 cell line	Krishnan and Maru, 2004
HAA	Black and green tea extract	Antimutagenic		*S. typhimurium*	Stavric et al., 1996
Mitomycin and UV	Black tea extract	Antimutagenic	Co-mutagenic effect as such, but antimutagenic in presence of liver enzymes		Imanishi et al., 1991
9AA, MNNG and Folpet	Galic acid	Antimutagenic	Blocking the membrane transporters of the mutagen		Roy et al., 2001
MeIQX	Black tea extract	Antimutagenic		*S. typhimurium*	Apostolides et al., 1996
4 NQO and UV, Gamma rays	Tannic acid	Antimutagenic	Activation of excision repair system	*E. coli* (WP2)	Sharma and Rao, 2009
B(a)P	Black tea extract	Antimutagenic	Prevention of DNA damage to germ cells	Swiss albino mice	Imanishi et al., 1991
PHIP	Caffeine	Antimutagenic	Competitive inhibition of Cyp450 through non-covalent interactions		Apostolides et al., 1996
MNNG	Black and green tea extract	Antimutagenic		*E. coli* (WP2)	Sharma and Rao, 2009
IQ	Caffeine	Antimutagenic	Inhibition of CyP450 isozymes, quenching free radicals and electrophiles	Rats	McArdle et al., 1999
B(a)P	Black tea polyphenols	Antimutagenic	Inhibition of CyP450 isozymes	Prevention of DNA adduct formation	Krishnan and Maru, 2004
PHIP	Black and green tea extract	Antimutagenic	Promotion of DNA excision repair system activity	Chinese hamster cells	Kuroda and Hara, 1999
B(a)P	TFs and TRs	Antimutagenic, anti-clastogenic		Mouse bone marrow cells and *S. typhimurium*	Krishnan and Maru, 2004

Heterocyclic aromatic amines (HAAs) are well-known mutagens found in meat during heat processing. Black tea has been shown to inhibit the mutagenic effects of HAAs in *S. typhimurium* (Stavric et al., 1996). On the other hand, it has been also reported that black tea extract at low concentration enhances the mutagenicity of 2-amino-3,4,7,8-tetra methyl 3H imidazo (4,5-f) quinoxaline and 3-amino-1-methyl-5H-pyrido (4,3-b) indole (Trp-P2) (Weisburger et al., 1996). Caffeine, a constituent of black tea, decreases the mutagenic potential of benzo(a) pyrene, 1,2-dibromoethane and 2-nitropropane in rat liver. However, no inhibitory effects were observed against 1-nitropyrene and 2-chloro,4-methyl thiobutanoic acid (Jolivette et al., 1998). Black and green tea extracts have been reported to possess a strong anti-mutagenic activity against food mutagens such as 2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline (MeIQX) and 2-amino 3-methyl imidazo (4,5-f) quinoline (IQ) (Krul et al., 2001). McArdle and colleagues (1999) showed that caffeine inhibited the mutagenicity of IQ. Later on, Yoxall et al. (2004) reported the anti-mutagenic activity of black tea on IQ due to induction of hepatic Cyp1a2 (cytochrome 450 enzyme) activity. They also observed the inhibition of colonic crypt foci formation induced by heterocyclic amine.

Exposure of Chinese hamster cells to tea tannins in the presence of S9 mix (liver enzymes) resulted in decreasing chromosomal aberrations induced by mitomycin and ultraviolet (UV) radiation mediated via promotion of DNA excision repair activity (Imanishi et al., 1991). Black tea extract, TFs, and TRs exhibit anti-clastogenic activity against cyclophosphamide (CP) and dimethyl-benz(a)anthracene (DMBA) in mouse bone marrow (Gupta et al., 2001). Shukla and Taneja (2001) observed that black tea extract prevented B(a)P-induced DNA damage in Swiss albino mice germ cells. TFs and gallated catechins strongly inhibited the activity of cytochrome P450 as compared with catechins (Apostolides et al., 1996). Addition of black tea extract was observed to inhibit the formation of heterocyclic amines, including 2-amino1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP) and MelQx, important food-borne pro-carcinogens produced during frying or broiling of meat and fish. Interestingly, decaffeinated tea was less effective than normal tea because of inhibitory effect of caffeine on cytochrome P450 system (Kuroda and Hara, 1999). Black tea selectively activates cytochrome P450 isoforms such as CYP1A2, CYP4A, and CYP2B in rat liver (Bu-Abbas et al., 1999). These activated cytochrome P450 isoforms enhance the bio-activation of pro-carcinogens. Lin and co-workers have demonstrated that black tea prevents DNA fragmentation formation by inhibiting direct reduction of PhIP (Kuroda and Hara, 1999).

Anti-cancer activity

A number of studies have shown that the black tea phytochemicals exert their chemopreventive effects directly or indirectly through the inhibition of tumor promoters. In the hamster buccal pouch carcinogenesis model, black tea extract showed chemopreventive effect by inhibiting DMBA-induced carcinogenesis (Chandra Mohan et al., 2005). Authors have also documented that this effect is associated with the inhibition of oxidative stress, formation of neoplastic lesions, activity of carcinogen metabolizing enzymes, and the incidence of bone marrow micronuclei by TFs and TRs. Moreover, black tea was also found to inhibit tumor invasion and cell proliferation. These data suggest strong inhibition of cancer development at different stages by administration of black tea. However, this potential of black tea was not always related to a lower incidence of cancer in epidemiological investigations. On the other hand, black tea did not appear as a cancer-promoting agent.

Aflatoxin belongs to the class of naturally occurring mycotoxins and food contaminants having potential carcinogenicity. The oral administration of aflatoxin caused reduction in the contents of DNA, RNA, protein, and glycogen, while the levels of cholesterol and phosphorylase activity were significantly increased in mice liver. Co-treatment with black tea extract was found to reverse these changes (Jha et al., 2012). Growth of human prostate carcinoma cells was recorded to inhibit when treated with TR plus genistein in the ratio of 1:40. However, TR alone did not show any significant inhibitory effect on cell growth. A reduced mammary gland tumor burden was observed in DMBA-treated female Sprague Dawley rats fed with a fat-rich diet (Gupta et al., 2001). However, this effect disappeared in rats fed with the AIN-76A diet. TFs were found to inhibit the proliferation of prostate cancer LNCaP cells, and this inhibitory effect varied in the following order: TF4 > TF3 > TF2 > EGCG > TF1. TF4 suppresses 5α-R1 activity that leads to the growth inhibition of androgens sensitive LNCaP cells. TFs and TRs also quench the FRs generated by testosterone, a promoter of prostate cancer.

Black tea has been found to reduce the mitotic index by three-fold (Sharma and Rao, 2009). Treatment of DMH (1,2-dimethyl hydrazine)-treated F-3-4-4 rats with black tea extract enhanced the activity of GST and reduced quinone reductase activity in colorectal epithelium. The drinking of black tea causes inhibition of nitrosomethyl benzamine-induced esophageal tumors by 70% in male Sprague Dawley rats (Wang et al., 1995). A single dose of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (103 mg/kg body weight) causes rapid proliferation in bronchiolar cells and tumor formation in the lungs of female A/J mice. However, treatment with 0.1% and 0.3% solutions of TFs inhibited these events (Sharma and Rao, 2009). Treatment with TFs also reduces the damage of homocysteine-injured human vascular endothelial cells (HUVECs) and inhibits homocysteine-induced oxidative DNA damage induced by quenching the FRs (Wang et al., 2012a). Black tea inhibited inflammation induced by 12-O-tetradecanoyl phorbol-13-acetate in the SENCAR mouse skin carcinogenesis model (Katiyar and Mukhtar, 1997a).

Numerous investigations have pointed out the contradictory results reported in the research concerning the drinking of black tea. Chronic treatment by black tea was not found to affect the progression of Peyer's patch carcinomas in colon induced by azoxymethane (AOM). Black tea has not shown any effect on the incidence and multiplication of colon cancers, and rather it was found to stimulate the growth of exophytic carcinomas (Weisburger et al., 1998). Some studies indicated that tea drinking increases the risk of bladder cancer (Lu et al., 1999). Later on, Zeegers and coworkers (2001) mentioned in their review that tea drinking inhibits bladder cancer and its proliferation. However, there are several in vitro and in vivo studies in which potent anti-cancer effects of black tea have

been reported. Administration of black tea extract suppresses intestine cancer in male F-3-4-4 rats. Black tea also increases the apoptotic index of tumors from 2.92±0.25 in controls to 4.13±0.46 in rats. After decaffeination, black tea extract was observed to reduce the number of lung tumors in NDEA-treated male C3H mice. Aqueous extract (4%) of black tea inhibited the development of pulmonary tumors in Swiss albino mice induced by NDEA (Sharma and Rao, 2009).

Black tea and green tea are potent antioxidants that demonstrate anti-cancer effects. Exposure of hepatoma AH109A and L929 tumor cells to TF1 and their gallates reduce their proliferation and invasion, while normal cells were found as unaffected (Zhang et al., 2001). Addition of EDTA, a chelator of antioxidants' anticancer effect of black tea disappeared, which suggests its strong antioxidant property (Zhang et al., 2001). Aqueous extract of black tea was also examined as an effective inhibitor of skin cancer induced by 7,12-dimethyl benz(a) anthracene in female CD-1 mice by inducing cell death and apoptosis (Kalra et al., 2005). Similarly, drinking of black tea was associated to inhibit the growth of papilloma by 35–40%. Treatment with decaffeinated tea has been shown to decrease papilloma growth in one case, and increase it in two others. Black tea treatment was shown to suppress the 70% growth of skin cancer induced by 30 mJ/cm^2 of UV-B light in SKH-1 female mice (Sharma and Rao, 2009). Black tea extract in a concentrated form ranged from 0.1–0.2 mg/mL and inhibited tumorigenesis in NDEA-treated male C3H mice by decreasing the number of hepatic tumors. Moreover, chemopreventive effect of black tea was shown to result from reduced DNA replication in HT rat hepatoma cells (Lea et al., 1993).

Inhibition of DNA replication and cell proliferation was observed in mouse erythroleukemia DS19 cells when treated with ethyl acetate extract of black tea. Treatment of JB-6 mouse epidermal cells with TFs has been shown to inhibit UVB-mediated activation of AP-1 (Nomura et al., 2000). Pre-treatment with black tea extract also decreased the number of skin papillomas induced by UV A+B light in hairless mice. The effect of black tea was higher than green tea (Record and Dreosti, 1998). Exploiting the anticancer property of black tea, plenty of preparations have been formulated. For example, a medicinal formulation having rhubarb, Fangdou tea, Dragon's bone, and premium black tea treats burns and scalds (Sharma and Rao, 2009). Therefore, tea can be used for preparing anti-cancer tea, powder, pill, capsule, anti-cancer-medicated wine, anti-cancer beer, liquid medicine, sweets, and anti-cancer biscuits (Sharma and Rao, 2009).

Molecular targets for cancer prevention

Intake of black tea has been reported to prevent various types of cancers. Extensive research in the last two decades has documented that black tea contains cancer preventive phytochemicals that modulate various cellular signaling pathways that can potentially be used not only for the prevention but also for the treatment of cancer (Fig. 4; Mikutis et al., 2013). TFs have been shown to display affinity to all of the selected cell nuclear structures such as histone proteins, double stranded DNA, and quadruplex DNA, thereby demonstrating a degree of unexpected molecular promiscuity. Most notably, TF4 exhibited the highest affinity with quadruplex DNA reported than reported by other phytochemicals so far. This study indicates the chemopreventive effect of black tea against cancer and its possible role as "life span essential."

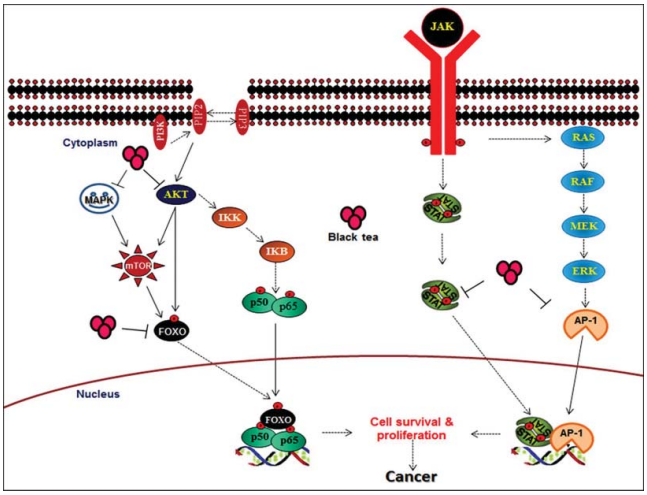

Figure 4. Oncogenic signaling pathways regulated by black tea polyphenols for prevention and therapy of cancer. Most human cancers are driven by chromosomal translocations or other genetic alterations that directly affect the functioning of cell cycle regulating proteins. Cell signaling pathways such as JAK, AKT/PI3K/mTOR, MAPK, EGFR, STAT, AP-1, FOXO, p50, and p65 contribute cell survival and proliferation. Black tea polyphenols are reported to inhibit deregulated cellular proliferation, de-differentiation, and progression by controlling cell proliferative pathways and by inducing apoptotic cell death. Blunt headlines indicate the inhibition of molecular targets by black tea polyphenols. The purpose of this mechanism is to present an appraisal of the current level of knowledge regarding the chemopreventive potential of black tea polyphenols via an understanding of their mechanism of action at the level of cell cycle regulation.

Growth factors, including insulin like growth factor (IGF), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF), are proteins that bind to receptors on the cell surface, thereby activating cellular proliferation. Aberrant expression of IGF-1 leads to increased cell proliferation, suppression of apoptotic signals, and invasion contributing to metastasis (Sharma and Rao, 2009). Black tea and its TFs inhibit IGF-1-induced progression of cells into S phase of cell cycle in prostate carcinoma DU145 cells, indicating that it has potential to disrupt the autocrine loops that are examined in several advanced cancers (Klein and Fischer, 2002). TFs also reduce mitochondrial membrane potential and induce ROS generation, p53 expression, Bax/Bcl-2 ratio, cytochrome-c release, cleavage of procaspase-3 and procaspase-9, and poly (ADP-ribose) polymerase (PARP) (Singh et al., 2011c). TF treatment suppressed the activation of AKT and NF-κB by suppressing phosphorylation, cyclooxygenase-2 (COX-2), and degradation of inhibitor of $\kappa B\alpha$ and $\kappa B\beta$ subunits. In addition, cyclin D1 expression (a transcriptional target of NF-κB) was also significantly down-regulated by TF.

Apoptosis is a normal physiological process that allows the removal of abnormal cells engaged in sustaining homeostasis in a living system (Singh et al., 2011b, 2012). Consequently, regulating apoptosis may prove to be a useful approach for prevention and therapy of cancer. Apoptosis-dependent inhibitory action of tea polyphenols has been found to suppress the growth of rat hepatoma, mouse erythroleukemia, and the growth of several human cancer cell lines, including MCF-7

breast carcinoma, HT-29 colon carcinoma, A-427 lung carcinoma, and UACC-375 melanoma (Katiyar and Mukhtar, 1997b). Anandhan et al. (2013) found that oral treatment of TF1 (10 mg/kg) attenuated 1 methyl,4 phenyl–1,2,3,6-tetrahydropyridine-induced neuro-inflammation and apoptosis through regulation of IL-1β, IL-6, TNFα, IL-10, glial fibrillary acidic protein, Bax, and Bcl-2. These authors also reported that the effect of black tea was found to be at par with that of green tea. Pre-exposure to black tea extract significantly decreased radiation-induced loss of cell viability, generation of ROS, mitochondrial dysfunction, activation of caspase-3, and apoptosis in normal lymphocytes compared with K562 cells. Black tea extract also regulates the activity of endogenous antioxidant enzymes. Changes in the mRNA expression of Bax, Bcl2, p53, and Nrf2 were also followed to assess regulation of radiation-induced apoptosis by black tea extract (Ghosh et al., 2014). TUNEL and DNA fragmentation assays indicated induction of apoptosis by hydro-methanolic and black tea in oral squamous cell carcinoma KB cells (Aghbali et al., 2014).

Activated AKT induces cell proliferation by regulating the downstream targets such as Wnt/β-catenin, cyclin D1, forkhead transcription factor 1 (FOXO1), cylcin-dependent kinase (CDK), p19, p21, and p27. Halder et al. (2012) found that TF1 and TR abrogated the activation of AKT pathway and its downstream targets. The increased expressions of p19, p21, and p27 and decreased levels of CDK2, CDK4, CDK6, and cyclin D1 were also observed. Cyclooxygenases, lipoxyenases (LO), and CYP450 regulate the metabolism of arachidonic acid, which is a well acceptable phenomena to reduce the risk of colorectal cancer (Fig. 3). Prostaglandin G2 (PGG2) formed by the action of COX-2 is involved in rapid cell proliferation in tumors, mitogenesis, invasiveness, and angiogenesis. COX-2 has been shown to be over-expressed in colorectal cancer cells. Interestingly, TFs were found to increase PGE2 formation by stimulating the COX-2 activity; however, catechins reduced the activities of both COX-1 and COX-2, and thus catechins had anticancer activity (Sharma and Rao, 2009). Numerous studies have reported that TFs exert anti-cancer effects by reducing cell survival and inducing apoptosis in different human cancers (Lu et al., 2000; Yang et al., 2000). These include 33 BES, 21 BES, SV40-WI 38, and Caco-2. Of these, TF3 (25 μM) had very strong anticancer effect through down-regulation of phosphorylation of c-jun, which lowers AP-1 activity and suppresses the growth of cancer cells. Co-treatment of TF2 and TF3 suppresses the growth of colon cancer cells by inhibiting the COX-2 activity and inducing apoptotic cell death.

Ehrlich's ascites carcinoma (EAC) affects the host's immune system by reducing the number of splenic lymphocytes. Black tea extract has been shown to induce p53-dependent apoptosis and inhibit Bcl2, thereby increasing the Bcl2/Bax ratio. TFs and EGCG induction of apoptosis has been repeatedly reported to be accompanied by enhanced hydrogen peroxide production in H661 cells. Mazumdar et al. (2013) have very recently reported that TFs successfully induce apoptosis in human medullary thyroid carcinoma (MTC) cell line by inversely modulating two molecular pathways: (i) stalling PI3K/AKT/Bad pathway that resulted in mitochondrial transmembrane potential (MTP) loss, cytochrome-c release, and activation of executioners caspase-9 and caspase-3, and (ii) upholding p38 MAPK/caspase-8/caspase-3 pathway via inhibition of Ras/Raf/ERK. Black tea extract and TFs treatment of KATO III human stomach cancer cells resulted in the induction of DNA fragmentation, a classic sign of apoptosis. In leukemic cancer cell lines (HL-60 and K-562), black tea has been shown to inhibit cell growth and induce apoptosis (Kundu et al., 2005). These effects were accompanied by increased expressions of caspases and Bax. Katiyar and Mukhtar (1997b) also reported cancer preventive potential of black tea via inhibition of tumor promoters, including ornithine decarboxylase, COX, LOX, and FR production. TF4 causes concentration-dependent inhibition of proliferation of A431 cells and mouse NIH3T3 fibroblasts. This effect was associated with the inhibition of mitogenic signal transduction, NF-κB activation, NO production, and auto-phosphorylation of epidermal growth factor receptor (EGFR) (Liang et al., 1999). TF1 has also inhibited the expressions of IL-6, monocyte chemo-attractant protein-1, and intercellular adhesion molecule-1 in bone marrow-derived macrophages through inhibition of NF-κB/MAPK pathways.

Black tea extract prevents formation of DNA adducts by inhibiting activity of CYP450 isozymes, namely CYP1A1, CYP1A2, and CYP2B1 (Krishnan and Maru, 2004). Feng et al. (2002) also found out that black tea decreased CYP450 1A1 activity in human hepatoma Hep G2 cells. TF treatment blocks GSH-Benzo(a) pyrene conjugation via enhancing GST level, thereby resulting in decreasing DNA adducts formation in tumor cells (Sharma and Rao, 2009). The down-regulation of AP-1, the transcription factor which is an association of the products of proto-oncogenes, such as fos and jun, is therefore considered to be a sound therapeutic target against cancer (Singh et al., 2011b). Black tea and green tea decreases AP-1-dependent cell proliferation in the mouse epidermal H ras mutant JB 6 cells, thereby inhibiting JNK1 (Chung et al., 1999). Suppression of EGF- or 12-O-tetradecanoyl-phorbol-13-acetate-induced transformation was observed when mouse epidermal cells were treated with TFs and EGCG. This effect was associated with the inhibition of AP-1 transcriptional activity and DNA binding affinity by tea phenols (Dong et al., 1997). Anti-metastatic effect of black tea extracts system was studied in oral squamous SCC-4 cells (Chang et al., 2012). The complete inhibition of invasion of cells was observed via the suppression of levels of p-FAK, p-paxillin, MMP-2, and uPA. In addition to these effects on gene expression, black tea extract also inhibited tumor growth in xenografted nude mice.

Clinical studies

Limited number of human intervention studies done on regular intake of black tea demonstrate its chemopreventive potential, while a substantial number of clinical investigations with green tea are documented. In vitro and preclinical studies support anti-cancer activity of black tea; however, its effect in human trails is uncertain. Two large prospective cohort studies of men and women found no association between the consumption of caffeine-rich tea and colon or rectal cancer (Gardner et al., 2007). Ganmaa et al. (2008) also observed similar effect between drinking of black tea and breast cancer. This supports a previous meta-analysis, which analyzed eight breast cancer studies and recorded no steady association with intake of black

tea (Gardner et al., 2007; Nie et al., 2014). Of the three newer investigations on ovarian cancer, only one study showed a significant protective association with tea drinking with intake of two or more cups per day (Baker et al., 2007). One case control study of 770 subjects with renal cell cancer found no relationship with tea consumption.

Studies on endometrial and skin cancer were also examined, and outcomes revealed significant inverse association between drinking two cups of black tea per day and cancer risk. In a cross-sectional study of postmenopausal Chinese women, the effect of drinking black tea was observed on the levels of estrogen and endostenedione in plasma. Higher levels are associated with a higher risk of breast cancer. The oestrone level was found to be 13% lower in green tea drinkers, compared with non-tea drinkers. However, in black tea drinkers, the levels were found to be 19% higher (Gardner et al., 2007). The authors concluded that this observation needs further study, because the number of participants involved was very low.

Recent epidemiologic studies, especially cohort and case-control studies, have yielded inconsistent findings regarding association between tea consumption and risk of lung cancer. Consumption of black tea was associated with lower incidence of rectal cancer in the population of Moscow. A pooled analysis of 13 prospective studies of renal cell cancer (n = 1480 cases) was carried out. The effect of black tea was observed more in women than men, possibly because of high alcohol intake by men. Black tea drinking and the incidence of rectal cancer were inversely correlated in a dose-dependent manner, and a minimum consumption level of about 80 g/month was required to have any significant effect. (Gardner et al., 2007). In contrast to the findings of the case control study mentioned above, taking three cups of black tea per day was associated with a 15% reduction in cancer risk as compared with drinking less than one cup per day. Very recently, Cassidy et al. (2014) selected 171,940 Nurses' Health Study and Nurses' Health Study II participants to examine associations between intake of total flavonoids and their subclasses and risk of ovarian cancer by using Cox proportional hazards models. They concluded that the higher intake of flavonols and flavanones as well as black tea consumption reduce the risk of ovarian cancer. A meta-analysis of case-control and cohort studies showed both green tea (RR: 0.75; 95% CI: 0.62–0.91) and black tea (RR: 0.82; 95% CI: 0.71–0.94) significantly associated with reduced lung cancer risk (Wang et al., 2014). The overall odds ratio (ORs) for coffee, green tea, and black tea intake examined recently by Bai et al. (2014) were 1.17 (95% CI: 1.03–1.33), 0.76 (95% CI: 0.66–0.95), and 0.80 (95% CI: 0.65–0.97), respectively. They suggested that greater consumption of fluid might have a protective effect on bladder cancer in Asian people.

A meta-analysis of lung cancer risk observed in 22 studies found no overall significant association with black tea drinking (Tang et al., 2009). No significant anti-cancer effect of black tea was observed on stomach, colorectal, lung, and breast cancers in a cohort study conducted in the Netherlands (Gardner et al., 2007). A systematic review proposed that black tea has a small beneficial effect on lung cancer risk in those who had never smoked. However, a meta-analysis of studies (n = 25) documented reported no significant relationship between black tea intake and colorectal cancer risk. In conclusion, although in vitro studies designate a number of possible mechanisms to elucidate how black tea and its bioactive phytochemicals could help to prevent cancer, further research from long-term human studies is needed to determine real life relationships with routine black tea drinking and incidence of cancer (Gardner et al., 2007).

Bioavailability and bioactivity

Subsequently, natural products differ in their bioavailability and bioactivity; these are most critical issues when assessing them for the development of functional foods, biopharmaceuticals, and nutraceuticals. Certainly, some sources demonstrating antioxidant properties in in vitro system may have no effect when consumed. This can be due to differences in the size of bioactive phyto-molecules, which influences the absorption of phytochemicals in the gut, and how different phytochemicals are processed in the body after absorption. However, intake also plays a key role in evaluating health value, with the frequently taken black tea potentially making a greater contribution to health beneficial phytochemical status among tea-drinking populations than the more bioavailable, however irregularly taken green tea. An added consideration for black tea is that the processing readily oxidizes the absorbed catechins into potentially less bioavailable TFs and TRs. Widlansky et al. (2005) recorded the higher levels of catechin (33%) in plasma after consuming 450 mL of black tea per day. However, after intake of 900-mL black tea per day for four weeks, catechins level in plasma was 29% more compared with baseline, indicating that the chronic consumption has no impact on the bioavailability of catechins.

Lack of a precise analytical technique to assess the presence of TRs and TFs in vivo can also make an underestimation of the bioactivity of black tea compared with green tea. However, since green tea and black tea display equal antioxidant activity in vivo in spite of containing different classes of flavonoids, it is supposed that at least some of the TFs and TRs are absorbed. A very limited evidence from clinical investigations exists suggesting that the flavonoids, namely TFs and TRs, in black tea are sufficiently bioavailable to stimulate the anticancer and anti-mutagenic effects described previously. The effects of food matrix on the bioavailability of anti-mutagenic compound of black tea were studied, and reduced activity was observed when milk was added to them (Widlansky et al., 2005). Maximum inhibition of 22, 42, and 78% was recorded in the presence of whole milk, semi-skimmed milk, and skimmed milk, respectively. A homogenized breakfast added together with black tea extract completely escaped the anti-mutagenic potential of black tea. This was due to the precipitation of milk components with tea polyphenols. The capacity of tea flavonoids to reach key tissues was estimated by Henning and colleagues, who randomly assigned men awaiting a prostatectomy to consume 1.421 per day of black tea, green tea, or a soft drink for five days. Analysis of prostate samples after surgery indicated that the concentration of tea flavonoids was higher in men given to tea. There has been some discussion about whether the addition of milk to tea affects the bioavailability of flavonoids (Kyle et al., 2007). Plasma levels of catechins, quercetin, and kaempferol increased significantly after tea drinking, but were unaffected by the addition of milk.

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