Black Tea Polyphenols-Mediated *In Vivo* Cellular Responses During Carcinogenesis

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Abstract: Tea (*Camellia sinensis*), a popular beverage, is consumed worldwide. The biological activities and mechanism(s) of chemopreventive effects of green tea polyphenols (monomeric catechins) have been extensively studied, while similar information regarding newly formed major black tea polyphenols (BTPs-oligomeric, polymeric) is not available. Therefore, this review focuses mainly on compiling the evidence on chemopreventive efficacy of black tea extract (BTE) / BTPs and describing their mechanism(s) of anti-initiating, anti-promoting and anti-progressor action(s) in *in vivo* experimental systems. Overall, the mechanism(s) implicated in the BTPs-mediated inhibition are diverse and involve effects on multiple molecular pathways and genes.

Keywords: Carcinogens, *in vivo* models, tumorigenesis, black tea polyphenols, chemoprevention, anti-initiation, anti-promotion, anti-progression.

INTRODUCTION

Cancer is one of the deadly diseases, characterized by unscheduled and uncontrolled proliferation of a cell, with the potential to spread to other organs. Majority of human cancers are caused, mediated or modified by exogenous/endogenous environmental factors. Although dose and duration of exposure to exogenous/endogenous carcinogen(s) is one of the determining factors, that alone is not sufficient to explain exposure-related outcome, as majority of cancers result from complex interactions between environmental exposure(s) and genetic/acquired susceptibility or protective host factors. Elimination of known and established carcinogens from the environment, i.e. primary prevention of cancer, has proven to be rather difficult due to social, economic, political reasons (e.g. tobacco). Despite the tremendous advancement in understanding molecular and cellular basis of cancer and in current treatment modalities, the mortality rates (age-adjusted) for cancer have not declined in the past 50 years. Considering the progress and the past experience in dealing with health problems, prevention appears to be a practical, cost effective and achievable approach for the management of cancer. Cancer chemoprevention, a 'prescription' approach refers to slowing, blocking, or reversing the development of the disease by the administration of naturally occurring or synthetic compounds and is based on the multi-step nature of carcinogenesis.

CARCINOGENESIS AND CHEMOPREVENTION

Carcinogenesis is a complex, multi-step and multifactorial process characterized by at least three stages viz. initiation, an irreversible event, begins when the cells in normal tissues are exposed to carcinogen and their genomic DNA undergoes damage and subsequent fixation of the damage; promotion, where initiated cells expand to form an actively proliferating multi-cellular pre-malignant tumor cell population; progression, an irreversible process which produces a new clone of tumor cells with increased proliferative capacity, invasiveness and metastatic potential. Compounds that inhibit any stage of carcinogenesis and delay or prevent cancer are called as chemopreventive agents. Based on their mode of action, chemopreventive agents can be classified into two groups: blocking agents, which impede the initiation stage either by inhibiting the formation of carcinogens or preventing the carcinogens from reaching / reacting with macromolecules; and suppressing agents, which arrest or reverse the promotion and progression of cancer, mainly by affecting or perturbing crucial factors that control cell proliferation, differentiation, senescence or apoptosis. A number of natural and synthetic compounds have been shown to possess chemopreventive properties [1] and many dietary constituents, in a variety of foods and beverages consumed by humans are receiving increasing attention [2]. These agents have been shown to posses anti-initiation, and/or anti-promotion, and/or anti-progression activities in different *in vivo* experimental systems [3], and are under clinical trials [4].

Tea (*Camellia sinensis*, family Theaceae) is consumed worldwide and has been associated with many health benefits including the prevention of cancer and cardiovascular diseases [5, 6]. The popularity and some evidence of potential health benefits of tea in experimental studies have prompted a large number of investigations on the chemical constituents of tea and their biological activities.

TEA CONSTITUENTS AND THEIR BIOCHEMICAL PROPERTIES

Tea is an aqueous infusion prepared from the dried leaves, leaf buds and internodes of *Camellia sinensis*. De-

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Table 1. Polyphenol Content of Green and Black Tea [7, 8]

	Components	g % of dry solid extracted*	g % of total polyphenols content
Black Tea	Catechins	3-10	30
	Theaflavins	3-6	13
	Thearubigins/PBPs	12-18	47
Green tea	Catechins	30-42	90
	Theaflavins	-	-
	Thearubigins/PBPs	-	-

*% of solid extracted from black tea = 25-35%, PBPs = Polymeric black tea polyphenols.

pending on the manufacturing process, teas are classified into three major types: black tea, green tea and oolong tea. Out of total, 76-78% of tea produced and consumed is black tea, 20-22% green tea and 2% oolong tea. Tea leaves contain more than 700 chemicals, among which the compounds closely related to human health are flavanoids, aminoacids, vitamins (C, E and K), caffeine and polysaccharides. In green tea (non-fermented), the polyphenol oxidase (PPO) enzyme present in the tea leaves is inactivated at the initial stage of tea processing to prevent oxidation of the leaf polyphenols. Polyphenols are the most significant group of components in tea, especially the catechin group of the flavanols. The major green tea polyphenols are: (-)-epigallocatechin-3-gallate (EGCG), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), (-)-epicatechin (EC), (+)-gallocatechin (GC), (+)-catechin which together may constitute 30% of the dry leaf weight (Table **1**) [7, 8]. Among these, EGCG is the major polyphenol which accounts for 60% of total catechins and is believed to be the most protective agent in green tea. Oolong tea (semi-fermented) is produced by partial fermentation of the fresh leaves before drying. During the manufacture of black tea, a major proportion of monomeric free catechins in the fresh green tea leaf undergo PPO-catalyzed oxidative polymerization, to form oligomers-theaflavins (TFs) (500-1000 Da) and polymers–polymeric black tea polyphenols (PBPs) / thearubigins (TRs) (>1 kD) in a process commonly known as fermentation (Table **1**) [7, 8]. TFs are well characterized group of oligomeric compounds e.g. theaflavin (TF-1), theaflavin-3-gallate (TF-2a), theaflavin-3′-gallate (TF-2b) and theaflavin-3,3′-digallate (TF-3), where as TRs are a group of polymeric compounds and five different fractions (PBPs 1-5) have been isolated although poorly characterized [9-11] (for structures of BTPs see Fig. (**1**)). Comparative concentrations and types of polyphenols in green and black tea have been presented in Table **1** [7, 8]. The precise composition of black tea is markedly influenced by the nature of the green shoots used and by the procedures employed in subsequent processing. Average caffeine levels in both black and green teas are 3-4% on a dry weight basis.

Extensive research on green tea / polyphenols has resulted in voluminous literature and evidence that they possess anti-bacterial, anti-viral, anti-oxidative, anti-mutagenic, anti-clastogenic, anti-inflammatory and anti-carcinogenic properties [12, 13] and possess diverse mechanisms for observed chemopreventive actions [2, 8, 14]. The observed protective activity of green tea has been attributed to the powerful scavenging and anti-oxidative ability of monomeric catechins and their gallates [2, 8]. In contrast with green tea, similar evaluations of major BTPs are either not available or limited partly due to its lower content of biologically active monomeric catechins [6, 8] and partly because of poor structural characterization of major BTPs [10, 11]. Therefore, this review focuses mainly on compiling the evidence on chemopreventive efficacy of black tea extract (BTE) / decaffeinated black tea extract (DBTE)/TRs (PBPs, polymeric polyphenols)/TFs (oligomeric polyphenols) and describing their mechanism(s) of anti-initiating, anti-promoting and anti-progressor action(s) in *in vivo* experimental systems.

Studies employing different *in vivo* animal models have demonstrated anti-initiating and anti-promoting activity of BTE and its components in both spontaneous and carcinogen-induced tumor models (Table **2**). Anti-progressor and anti-immunosuppressive activities of BTE/polyphenols have also been demonstrated in *in vivo* experimental animals bearing transplanted tumors or xenografts (Table **3**). Oral and topical administration of BTPs has been shown to modulate incidence / multiplicity / latency period or other degenerative changes at various organ sites in experimental animals (Table **2**). The routes and doses employed in evaluation of *in vivo* chemopreventive effects of black tea and its different components were: drinking water [BTE (0.6-4%), polymeric black tea polyphenol rich extract (PBPE, 1.5%), caffeine (0.044-0.24%), TFs (360 ppm)], diet [polyphenon-B (0.05%)], topical [PBPs 1-5 (200 μg)] and i.p. [TFs (0.02 mg)]. It may be noted that the observed anti-initiation, anti-promotion and anti-progression activities have been shown at doses (per kg BW) much higher than regularly consumed by humans, although none of the studies covered in Table **2** have reported BTPs exposure-related toxicity. Most of these reports have not measured the circulating and tissue levels of BTPs and their conjugates or metabolites. Available pharmacokinetic evaluations in rodents show low nanomolar levels of monomeric catechins and oligomeric TFs in plasma probably due to poor systemic bioavailability / rapid metabolism and excretion while pharmacokinetics of TFs and TRs are less well characterized [15, 6]. The poor bioavailability of other plant polyphenols has also been reported [16]. As presented in Table **2**, several studies have been undertaken employing crude BTE, DBTE, TFs and caffeine to evaluate

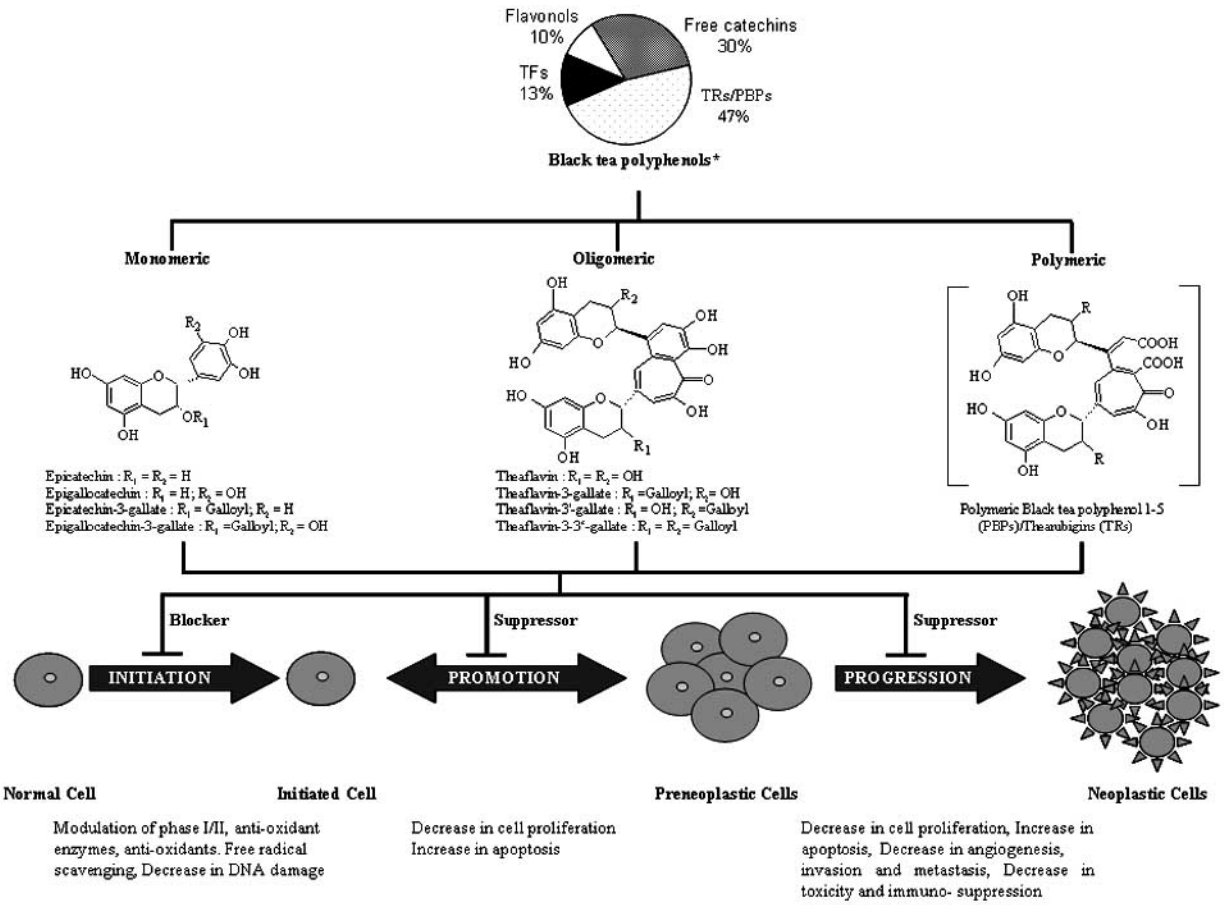

Fig. (1). Schematic diagram showing multistep process of carcinogenesis, steps at which black tea extract/polyphenols exhibit their effects and the black tea extract/polyphenols -mediated biological effects in experimental systems *in vivo*.

*levels (g) of different polyphenols/100g of total polyphenols isolated from black tea (for details see Table **1**).

their biological and chemoprotective activities, but similar efforts have not been received by TRs, despite the fact that these are the most abundant group of compounds in black tea. This is partly due to the difficulties in isolation of TRs and poor chemical characterization of these compounds. Several groups have not been able to achieve separation of TRs in spite of employing various modern chromatographic techniques [10, 11]. This is due to high affinities of these compounds for the matrix. Attempts at methylating or acylating the phenolic groups of TRs, which are responsible for their strong binding to active surfaces, have also met with limited success. Taking all these into consideration, TRs have been extracted employing the liquid-liquid extraction [9]. However, isolation by this method is very laborious, time-consuming, costly and not practical for scale up. While maintaining similar conditions, a logical and obvious approach, using the Soxhlet continuous extractor, has also been employed [11]. TRs isolated by both the methods have been shown to be free from caffeine, catechins and TFs. TRs have shown characteristic spots on acid hydrolysis suggesting proanthocyanidin nature, and also shown characteristic Fourier-transformed infra-red and Nuclear magnetic resonance spectra suggesting polymeric nature [11].

BTPs have shown the anti-oxidant properties that result from their ability to sequester metal ions and to scavenge reactive oxygen and nitrogen species [17]. These polyphenols are also known to bind to proteins which affect certain enzymes, receptors and membrane activities specifically resulting in different biological activities like stimulation of detoxification system through selective induction or modification of phase I and phase II metabolic enzymes, modulation of biochemical markers of tumor initiation and promotion etc. [2, 8]. Overall, the mechanisms implicated in the inhibition of tumorigenesis are diverse and appear to involve a combination of anti-oxidant, anti-inflammatory, immunomodulatory, pro-apoptotic and other properties of BTPs as well as its effect on genes and molecular pathways [18-20].

MECHANISM(S) OF CHEMOPREVENTIVE ACTIONS OF BTPs

BTPs can act as blockers and / or suppressors by inhibiting carcinogenesis at initiation and / or promotion stages.

MECHANISM(S) OF ANTI-INITIATING ACTION

Cellular metabolism plays a very crucial role in the process of initiation during carcinogenesis. Xenobiotics entering into the cellular environment are metabolized by xenobiotic metabolizing [phase I (functionalization) and phase II (conjugation)] enzymes (XMEs), rendering them into less toxic and more water soluble compounds. In all organisms, XMEs serve as an efficient defense mechanism against potential

Table 2. ***In vivo*** **Chemopreventive Effects of Black Tea Extract/Polyphenols/Constituents in Different Rodent Models**

S. No.	Animal Model	Organ	Carcinogen Dose / Route / Duration	Black Tea Extract / Polyphenols Dose / Route / Duration	Salient Observations	Ref.
Crude black tea extract						
1	SKH-1 mice	Skin	i) UV-A 1.8 x 10^{-3} W/cm^2, 5-15 mins/day, 5x/wk-30 wks ii) UV-B 2.4 x 10^{-4} W/cm^2, 5-15 mins/day, 5x/wk-30 wks	BTE 1.25-2%, sole drinking fluid, 1 wk prior till end	Decreased multiplicity, increased latency period of skin tumors	[79]
2	SKH-1 mice	Skin	i) UV-B 180 mJ/cm^2, 5 mins-1x/day -2 days ii) UV-B 180 mJ/cm^2, single	i) SBTE 0.8%, sole drinking fluid, 2 wks prior till end ii) SBTE, BTF1, BTF2 0.2 mg/ cm^2, 30 mins prior , topical	Decreased incidence, severity of erythema, skin fold thickness	[59]
3	SD rats	Mammary gland	i) DMBA 5mg, gavage, single on 49th day ii) IQ a) 8.4 mg, gavage, 2x/ 1st wk b) 14 mg, gavage, 2x/ 2nd-5th wks	BTE i) & ii) 1.25% (w/v), sole drinking fluid, 1 wk prior till end	i), ii) Decreased mammary gland tumor multiplicity, volume	[52]
4	Swiss mice	Lung	DEN 20 mg/kg BW, gavage, 1x/wk-8wks	BTE 1, 2, 4%, sole drinking fluid, simultaneously + 20 wks	Decreased incidence, multiplicity of adenoma / adenocarcinoma	[80]
5	A/J mice	Lung, Rhabdo-myosar-coma	Spontaneous tumors	i) BT 0.5, 1, 2%, sole drinking fluid, 60 wks ii) Instant BT 0.3-0.6%, sole drinking fluid, 52 wks	Decreased incidence, multiplicity, volume	[81]
6	i) CD-1 mice ii) SKH-1 mice	Skin	i) DMBA + TPA 200nmol, topical, single + 5nmol, topical, 2x/wk-24 wks ii) UV-B 30 mJ/cm^2, 25 secs, 2x/wk-22 wks	i) BT 1.25%, sole drinking fluid, 11 wks post papilloma development ii) BT 6 mg/ml tea solids, sole drinking fluid, 11 wks post papilloma development	i) Decreased papilloma growth ii) Decreased number of malignant, nonmalignant, tumors, BrdUr labeling, mitotic index, increased apoptotic index	[65]
7	SKH-1 mice	Skin	UV B 30 mJ/cm^2, 2x/wk - i) 35 wks, ii) 40 wks, iii) 45 wks	i) Caffeine 0.072, 0.24%, sole drinking fluid, 35 wks ii) LBT, LDBT 0.9% , sole drinking fluid, 40 wks iii) BT, DBT, DBT + Caffeine 1.25% , 1.25%, 1.25% +0.036%, sole drinking fluid, 45 wks	Inhibited UVB-induced complete carcinogenesis, DBT < BT, caffeine alone had significant effect, addition of caffeine to DBT restored inhibitory effect	[82]
8	SD rats	Liver	DAB 0.06%, diet, 12 wks	Polyphenon-B 0.05%, diet, 24 wks	Decreased pre-neoplastic, neoplastic lesions, oxidative stress. Modulation of XMEs	[27]
9	Syrian golden hamsters	Buccal pouch	DMBA 0.5%, topically, 3x/wk-14 wks	Polyphenon-B, BTF-35 0.05%, diet, 4 wks prior till end	Decreased tumor incidence	[73]
10	Syrian golden hamsters	Buccal pouch	DMBA 0.5%, topical, 3x/wk-14 wks	Polyphenon-B 0.05%, diet, 14 wks	Decreased incidence, multiplicity, tumor burden, hyperplasia, dysplasia	[25]
11	Syrian golden hamsters	Buccal pouch	DMBA 0.5%, topical, 3x/wk-14 wks	Polyphenon-B 0.05%, diet, 14 wks	i) Inhibited DMBA-induced genotoxicity, development of HBP carcinomas ii) Inhibited carcinomas by restoring normal cytokeratin expression, cell proliferation, induced apoptosis	[58], [26]
12	Mouse	Skin	DMBA + TPA 52 µg, topical, single + 5 µg, topical, 2x/wk	BTP i) 3 wks prior to DMBA application ii) prior to each treatment of TPA	Observed anti-initiating and anti-promoting activities	[83]
13	SKH-1 mice	Skin	UV-B 30 mJ/cm^2, 25 secs, 2x/wk-22 wks	BT, DBT (0.6%), Caffeine (0.044%), LDBT + Caffeine (0.6% + 0.044%), sole drinking fluid, 1 wk after UV treatments for 23 wks	Caffeinated black teas, caffeine decreased tumor multiplicity, parametrial fat pads, thickness of dermal fat layer, decaffeinated tea was inactive	[84]

(Table 2). Contd…..

S. No.	Animal Model	Organ	Carcinogen Dose / Route / Duration	Black Tea Extract / Polyphenols Dose / Route / Duration	Salient Observations	Ref.
14	A/J mice	Lung	NNK 103 mg/kg BW, i.p., single	BT 0.6%, sole drinking fluid, starting 16 wks after NNK treatment till 52 wks	Inhibition of tumor multiplicity, incidence	[66]
15	F344 rats	Colon, Small intestine	AOM 7.4mg/kg BW, s.c., 1x/wk-10 wks	BT 50 mg/kg,1 wk after AOM for 16 wks	Decreased tumor incidence, multiplicity	[74]
Decaffeinated black tea						
16	A/J mice	Lung	NNK 103 mg/kg BW, i.p., single	DBTE 0.3% or 0.6%, sole drinking water i) Starting 2 wk before, till 1 wk after NNK ii) Starting 1 wk after NNK treatment till end	Decreased tumor multiplicity without effect on incidence	[85]
17	C3H mice	Liver, Lung	DENA 50 µg/kg BW,i.p., 1x/wk-8 wks	DBT 1.25%, sole drinking fluid, 2 wks prior to & during 40 wks	Dose dependent decrease in multiplicity in lung and liver	[53]
18	SKH-1 mice	Skin	i)DMBA 200 nmole, topical, single ii)UV-B 30 mJ/cm^2, 25 secs, 3 wks after DMBA treatment, 2x/wk-31 wks	DBTE,BT 0.63 % or 1.25%, sole drinking fluid, 2 wks prior to during 31 wks	Decreased incidence, multiplicity, volume of keratoacanthomas, carcinomas	[86]
19	SD rats	Esophagus	NMBzA 2.5mg/kg BW,s.c., 2x/wk-5wks	DBT 0.6%, sole drinking fluid, during and after NMBzA treatments till 39 wks	Reduction in tumor incidence, mulitiplicity	[87]
Thearubigins						
20	Swiss bare mice	Skin	DMBA + TPA 20 nmole, topical, single + 1.8 nmole, topical, 2x/wk-40 wks	PBPs 1-5 200µg , topical 20 mins prior to TPA treatment	Decreased multiplicity, incidence of skin papillomas, cell proliferation markers, transcription factors at 10-40 wks	[56]
21	SD rats	Colon	DMH 40 mg/kg BW, s.c., 2x/wk-3wks	PBPE 1.5%, sole drinking fluid, 15 days prior till end	Inhibited colorectal carcinogenesis by decreasing tumor volume, multiplicity, inhibited cell proliferation *via* Wnt / β-catenin pathway	[40]
Theaflavins						
22	Mice strain A	Lung	B(a)P 4µg, s.c., single	TFs, EGCG and ECG 0.02 mg, 0.01 mg and 0.004 mg, i.p., till end	Restricted lung carcinogenesis by differential modulation of expression of p53, H-ras, c-myc, cyclin-D1	[60]
23	A/J mice	Esophagus	NMBA 3x/wk-5 wks, s.c.	TFs (360ppm), EGCG (1200ppm) sole drinking fluid, 2 wks prior till end	Decreased rate of tumor formation, only TFs decreased the multiplicity	[88]
24	A/J mice	Lung	NNK 100 mg/kg BW, i.p., single	TFs 0.1%, sole drinking fluid, 2 days after NNK treatment for 16 wks	Decreased tumor incidence, multiplicity	[89]

AOM = Azoxymethane; B(a)P = Benzo(a)pyrene; BrdUr = Bromodeoxyuridine ; BT = Black tea; BTE = Black tea extract; BTF1 = Ethyl acetate extractable; BTF2 = n-butanol extractable; BTF-35 = Black tea extract enriched with theaflavins and catechins; BTPs = Black tea polyphenols; BW = Body weight; DAB = ρ-Dimethylaminoazobenzene; DBT = Decaffeinated black tea; DBTE = Decaffeinated black tea extract; DEN = Diethyl nitrosamine; DMH = 1,2- dimethylhydrazine; DMBA = 7, 12-dimethylbenz (a) anthracene; ECG = (-)-Epicatechin-3-gallate; EGCG = (-)-Epigallocatechin-3-gallate; HBP = Hamster buccal pouch; i.p. = Intraperitoneal; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; LBT = Lyophilized black tea; LDBT = Lyophilized decaffeinated black tea; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NMBA = N-nitrosomethyl-benzylamine; PBPs = Polymeric black tea polyphenols; PBPE = Polymeric black tea polyphenols rich extract; Polyphenon-B = mixture of different catechins, caffeine, oligomers and polymers of tea polyphenols; SBTE = Standardized black tea extract; s.c. = Subcutaneous; TF = Theaflavins; TPA = 12-*O*-tetradecanoyl phorbol-13-acetate; UV-A = Ultraviolet light (320-375 nm); UV-B = Ultraviolet light (280-320 nm); XMEs = Xenobiotic metabolizing enzymes.

negative action of xenobiotics. Hence, these XMEs could be one of the probable targets for cancer chemoprevention.

a. Effects on Carcinogen-Activating Phase I Enzymes

Phase I enzymes predominantly cytochrome P450s (CYPs) are a diverse superfamily of haem-containing enzymes found in many organisms. Phase I enzymes play an important role in the very first step of metabolism, where xenobiotics are processed to more electrophilic moieties by addition of functional groups for further detoxification by phase II enzymes. Among the different reactions catalyzed by CYPs, hydroxylation at the vacant position of an aromatic ring is considered to be the hallmark for the initiation of carcinogenesis. These electrophilic moieties will further react with cellular macromolecules to form adduct, there by marking the process of initiation. Thus, decreased activation of carcinogens due to modulation of CYPs could be one of the main targets for prevention of the cancer initiation process.

Administration of BTE (2%) has been shown to increase the activities of CYP 1A1, 1A2 and 2B1 in rat liver while,

total CYPs, b5, NADPH-cytochrome c (P450) reducatse, CYP 2E1 and 3A4 were unaffected [21]. Administration of BTE (2%) and caffeine (0.04%, 0.055%) has been shown to induce the activity and the levels of CYP1A2 protein in rat liver. Induction of CYP1A2 was also noted after intragastric caffeine (100 mg / kg BW). It is note worthy that close correlation of the increase in CYP1A2 was observed only with plasma caffeine levels but not with combined tea polyphenols [22, 23]. Administration of English breakfast tea resulted in enhancement of activity of CYP 1A1 and 1A2 in rat liver microsomes while activity of CYP2D, 2E and 3A were unaltered [24]. Dietary polyphenon-B (0.05%, mixture of different catechins, caffeine, oligomers and polymers of tea polyphenols) has been shown to decrease (i) the dimethylbenz (a) anthracene (DMBA)-induced increase in the levels of total CYPs in buccal pouch and liver of hamsters [25, 26], (ii) p-dimethylaminoazo-benzene (DAB)-induced levels of total CYPs, CYP 1A1 and activity of CYP1A1, 1A2 and 2B [27] and (iii) DMBA-induced levels of total CYPs and cytochrome b_5 [28]. Notably, polyphenon-B alone did not alter the basal levels of CYPs and their activities [25-28]. Oral administration of BTE (1%) induced the activity and the levels of CYP1A2 protein in rats leading to more rapid metabolism of heterocyclic amine [29, 30]. Exposure to DBTE (2.5%) or PBP mix (PBPs 1-5) (1%) significantly decreased the benzo(a)pyrene [B(a)P]-induced CYP1A1 and 1A2 activity as well as protein levels in liver and lungs of mice. Administration of DBTE or PBP mix alone did not alter the basal levels and activities of CYP1A isozymes [31]. Oral administration of TFs (20 mg / kg) and theafulvins (TFu) [(2 mg / kg)-water soluble fraction of TRs] decreased the activity of CYP1A1 and the levels of CYP1A1, 2E1, 3A in rat intestine, while the levels of total CYPs and activity of CYP isozymes in liver were not altered [32]. Transplacental dibenzo[a,l]pyrene(DBP)-induced lung tumor multiplicity and mortality due to lymphoma was decreased by caffeine (0.09-0.27 mg / ml) in mice [33].

Considering the above mentioned observations on the effects of BTE / DBTE / PBPs / TFs / TFu / caffeine on the modulation of levels and / or activities of different CYP450 isozymes, role of BTE, BTPs in modulation of phase I metabolizing enzymes and their regulation needs comparative and detailed investigations.

b. Effects on Anti-Oxidant and Detoxifying Phase II Enzymes

Phase II enzymes play an important role in the detoxification of activated carcinogens by eliminating the reactive intermediates from cellular environment resulting in the decrease in the DNA and protein adducts. Phase II enzymes catalyze conjugation reactions such as glucuronidation, sulfation, methylation, acetylation and mercapture formation wherein phase I introduced functional groups are conjugated with endogenous, polar products (glutathione, glucuronic acid) rendering them less toxic and more water soluble. Therefore, chemopreventive agents altering the activity and / or levels of phase II enzymes would play an important role in blocking the initiation process. The expression of phase II enzymes is governed by a cis-acting regulatory element named the anti-oxidant response element (ARE). ARE containing gene is regulated by nuclear transcription factor erythroid 2 p45–related factor2 (Nrf2), a member of the cap 'n' collar family basic-leucine-zipper family of transcription factors *via* ARE. In an inactive state, the Nrf2 is sequestered in cytoplasm by kelch-like-ECH-associated protein 1 (keap 1), which in turn is bound to actin cytoskeleton. Upon activation, Nrf2 dissociates from keap1 and translocates to nucleus where it heterodimerizes with other leucine zipper proteins to transcriptionally activate the downstream genes [34].

Administration of BTE (2%) resulted in an increase in the UDP- glucuronosyl transferase (UDPGT) activity in rat liver, where as glutathione-*S*-transferase (GST) activity was unaffected [21]. Study with BTE and DBTE has shown that only BTE enhanced the activity of both GST and UDPGT enzymes. Comparative evaluation of green and black tea extract and DBTE suggests the role of newly formed products of oxidation of flavanols in black tea such as TFs and TRs in the observed enhancement in activity of phase II enzymes [35]. Oral BTE (1, 2 %) administration increased the activity of GST and glutathione peroxidase (GPx) in rat liver in both control as well as carcinogen controls [36]. Administration of BTPs (50 mg / kg BW) has been shown to decrease azoxymethane (AOM)-induced GST-P mRNA levels in rat colon. Rats treated with BTPs have greater γ-glutamyl cysteine synthetase (γ-GCS) expression in normal mucosa than that in control. Available evidence shows that BTPs modulate cyclooxygenase II (COX-2), inducible nitric oxide synthase (iNOS) and glutathione (GSH) related gene expressions in colon tumors suggesting that BTPs have possible chemotherapeutic activity [37]. Dietary polyphenon-B (0.05%) alone or in combination with DMBA significantly increased the activity of GST, γ-glutamyl transpeptidase (GGT) as well as DT-diaphorase in the buccal pouch and liver of hamsters [25, 26] and mammary glands of rats [28]. Study from the same group has shown that simultaneous treatment with polyphenon-B decreased the DAB-induced activity of GST while it enhanced the DAB-inhibited activity of quinone reductase in rat liver [27].

Effect of BTE as well as crude and purified components such as TFs and EGCG enhanced the activity of GST in mouse liver at 15 days when compared to carcinogen control [38]. Oral administration of BTE induced the levels of UDPGT enzyme in rats leading to more rapid metabolism and excretion of heterocyclic amine. Black tea inhibited the formation of heterocyclic amine-induced colonic aberrant crypt foci (ACFs) in the rats. Black tea administration resulted in altered urinary metabolite profile of 2-amino-3-methylimidazo [4, 5-*f*] quinoline (IQ) such that levels of parent compound and IQ-sulfamate were decreased with concomitant increase in glucuronide and sulfate conjugates. These results indicate that black tea possesses anticarcinogenic activity in the colon, and this involves multiple mechanisms [29, 30]. Pre-treatment with English breakfast tea (2%) and caffeine (0.0625%) did not alter the activity of UDPGT and GST enzymes in rat liver [23, 24]. Administration of BTE and DBTE did not alter the activity of GST-α, GST-μ and UDPGT in rats [39]. Evaluation of phase II enzymes in liver and intestine of rats treated with TFs (20 mg / kg) and TFu (2 mg / kg) showed no alterations in the activity of glucuronosyl transferase, GST, sulpho-transferase and epoxide hydrolase in liver and intestine except in intestinal

glucuronosyl transferase which was inhibited by TFu [35]. Consistent with these findings, PBPE has shown significant enhancement in the activity and the levels of GST and NAD(P)H quinone oxidoreductase-1 (NQO1) in liver and colon of rats [40]. It is noteworthy that rats pre-treated with PBPE and subsequently challenged with 1, 2-dimethylhydrazine (DMH) also showed enhanced activity and levels of total GST and isoforms and NQO1, suggesting increased detoxification of DMH by PBPE-induced phase II enzymes *in vivo* [40]. Pre-treatment with PBPE significantly increased the levels of total GST both in liver and lungs. Further study also showed increase in the levels of GST isoforms (α, μ and π) in liver, while in lungs only levels of GST-α were significantly increased. Enhanced level of another phase II enzyme, NQO1, in both liver and lungs of mice has also been demonstrated in PBPE treated control as well as carcinogen treated mice [34]. Parallel to this, increase in GST-α and NQO1 mRNA levels were found in both lung and liver of PBPE pre-treated mice. Further study showed PBPE-mediated increase in Nrf2 protein levels and enhancement in its nuclear translocation and DNA binding to GST and NQO1 ARE gene that paralleled the increased transcriptional up-regulation of GST and NQO1 respectively. However, the increase in total Nrf2 levels may be post-transcriptional as Nrf2 mRNA levels in both liver and lungs were unaltered by PBPE pre-treatment [34].

Under normal un-induced condition, Nrf2 is regulated by its cytosolic inhibitor keap1 and PBPE treatment resulted in increased protein level of keap1 in liver. A further study showed that it may be due to their increased stability and was not physically associated with Nrf2, which may indicate that Nrf2, upon PBPE pre-treatment, dissociates from keap1 protein and translocates to nucleus [34]. The study has also suggestive evidence for the potential role of protein kinase C (PKC) in the phosphorylation of Nrf2 and its binding partners. Taken together, pre-treatment with PBPE enhanced the Nrf2 protein levels, released Nrf2 from keap1, increased Nrf2 stability (by decreasing its ubiquitination in liver cells through post-translational modifications such as phosphorylation influenced by PKC), its nuclear accumulation and DNA binding resulting in increased enzyme activity which in turn, plays an important role in carcinogen detoxification.

Administration of BTE did not alter the levels of GSH and cysteine in the liver or kidney of rats, where as levels of GSH were increased in blood [21]. BTE and DBTE have been shown to decrease the catalase (CAT) activity, while activities of GPx and superoxide dismutase (SOD) were unaffected in liver [35]. Cigarette smoke condensate-induced oxidation and microsomal protein loss were prevented when the guinea pigs were given BTE (20 gm / L) [41]. Dietary polyphenon-B alone and in combination with DMBA has been shown to increase the levels of GSH, activity of GPx, SODs (Mn-SOD and Cu-Zn SOD), CAT and the ratio of GSH / GSSG in buccal pouch and / or liver when compared to control and carcinogen controls [25-28]. Oral administrations of BTE as well as crude and purified components such as TFs and EGCG have resulted in an increase in the activity of CAT in liver at 12 weeks, where as only TFs and EGCG were shown to increase DMBA-induced activity of GPx and SOD at 15 days of treatment [38]. Exposure to BTE increased the levels of GSH, GSH / GSSG and oxygen radical absorbance capacity in rats [39].

c. Effects on Xenobiotics-Induced DNA Damage

Most environmental carcinogens entering into the cellular environment undergo bio-activation during which procarcinogens get converted to electron deficient reactive intermediate(s). These intermediate(s) in turn can form chemical adduct(s) with nucleophilic moieties in DNA, RNA and proteins [3]. In addition, changes like DNA single / double strand breaks, cross linkages, depurination / depyrimidation, dimerization of pyrimdines and structural modification of DNA bases / deoxyribose, etc. may also result. Products of oxidative damage to macromolecules have been identified in biological materials such as plasma, urine and tissue / blood cells and may serve as biomarkers for oxidative damage.

Administration of DBTE (0.6%) did not alter the levels of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced DNA methylation in mouse lungs, although a significant reduction in lung tumor multiplicity was observed [42]. Administration of BTE (1%) has been shown to reduce the levels of IQ-derived DNA adducts in liver of rats when compared to IQ alone, suggesting the role of BTE in facilitating the rapid metabolism, detoxication and excretion of IQ [43]. Pre-treatment with BTE (2%) inhibited 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-induced DNA adduct formation in mammary gland and several other tissues in mice, while it did not affect the rate of adduct removal [44]. Pre-treatment with BTE (1.25%) decreased the formation of AOM-induced DNA adducts in rat colon, where as in liver it was unaffected [45]. Dietary polyphenon-B (0.05%) decreased DAB-induced levels of 8-hydroxy 2-deoxyguanosine (8-OHdG) in liver of rats when compared to DAB and polyphenon-B alone [27]. Topical pre-treatments with PBPs 1-5 or PBP mix (200μg) decreased the levels of B(a)P-derived DNA adducts in mouse skin when compared to carcinogen control although the extent of decrease was different in various groups [46]. Oral treatments with TRs or TFu (40 mg/kg) decreased DMH-induced levels of 8-OHdG in colon mucosa of rats [47]. Exposure to BTE (5, 10, 20%) and single dose of TFs and TRs has been shown to decrease cyclophosphamide- and DMBA-induced chromosome aberrations (CAs) and sister chromatid exchanges (SCEs) in mice [48]. Similarly, in another study TFs (20, 40, 80 mg / kg) and TRs (40, 80, 160 mg / kg) have been shown to posses anticlastogenic effects as seen by the decrease in B(a)P-induced CAs and SCEs [49]. Administration of TFs has been reported to decrease the levels of the malondialdehyde-DNA adducts (M_1dG) in mammary tumors of transgenic mice, while the levels of M_1dG in leukocytes were not influenced by TFs [50].

In several studies, oral BTE (1, 1.25, 2 %) administration significantly decreased the AOM-induced ACFs in rats [36, 51, 52]. Similarly DBTE (0.63, 1.25 %) has been shown to decrease the diethylnitrosamine (DEN)-induced hepatic foci in mice [53]. The observed decrease in pre-neoplastic marker foci probably reflects decrease in carcinogen-induced DNA damage and initiation of carcinogenesis.

The observed BTE / BTPs (TFs, TFu, TRs)-mediated decrease in the levels of carcinogen-derived DNA adducts

could be due to inhibition of phase I enzymes / enhancement of phase II enzymes (decreased formation of DNA adducts); enhanced repair of adducts; turnover of adduct containing cells; direct scavenging of reactive species by BTE / BTPs; or dilution of adducted DNA by newly synthesized non-adducted DNA in response to chemopreventives. Studies so far have shown BTPs-mediated inhibition of carcinogen-induced phase I enzymes and enhancement of the phase II enzymes. Hence, convincing evidence on other aspects e.g. enhanced DNA repair, apoptosis of adduct containing cells etc., if responsible for decreased DNA-adduct levels, needs attention.

MECHANISM(S) OF ANTI-PROMOTING ACTION

The tumor promotion involves the clonal expansion of initiated cells to give rise to tumor comprised of pre-neoplastic cells. This stage is largely characterized by two important cellular events viz. cellular proliferation and apoptosis. Evidence suggests that in response to various extracellular stimuli, cellular kinases including PKC, PI3 Kinase, mitogen-activated protein kinases (MAPKs) [extracellular signal-regulated kinase (ERK), p38, c-Jun N-terminal kinase (JNK)] are activated, which in turn regulate the transcription factors [jun, fos, nuclear factor kappa B (NF-κB)] thereby modulating the downstream effector molecules associated with various cellular responses such as cell proliferation, inflammation, differentiation, apoptosis, etc. This therefore suggests that modulation of these signaling effector molecules to either suppress cell proliferation or to induce apoptosis would be one of the important strategies by which a potential chemopreventive could inhibit promotion phase of carcinogenesis.

a. Effects on Cellular Kinases

Tumor promotion is an important process in carcinogenesis and 12-O-tetradecanoylphorbol 13-acetate (TPA) has been used as a model tumor promoter. Studies have shown that primary site of action of TPA is PKC, located on cell membranes. PKC plays an important role in transducing the signal from mediators across the membrane and in tumor promotion [54, 55]. It has also been shown that TPA induces cell proliferation by decreasing activation of signaling kinases (c-Jun, ERK, p38, Akt), transcription factors [activator protein-1(AP-1), NF-κB] and inflammatory protein (Cox-2). Therefore, an agent that is able to block tumor promoter-mediated cellular responses is likely to be an effective anti-tumor promoter. Pre-treatment of mouse skin with PBPs has been shown to decrease the TPA-induced phosphorylation of ERK and p38 during tumor development, while expressions of total ERK and p38 were unaltered [56]. Similarly, pre-treatment with PBPE has been reported to inhibit DMH-induced activation of ERK and JNK [40]. A report from the same group has shown that activation of PKC is important for PBPE-induced release of Nrf2 from keap1 and stabilization of Nrf2 [34]. However, no reports on the effects of pre-treatments of BTPs on TPA-induced PKC *in vivo* are available. These findings thus suggest that PBPs modulate cellular kinases *in vivo* to exert its chemo-protective effects [56]. Further studies on the *in vivo* effects of BTPs on signaling kinases are needed.

b. Effects on Transcription Factors and Oncogenes

AP-1 family of transcription factors is composed of homo- and hetero-dimers of the Fos (c-Fos, FosB, Fra-1, Fra-2) and Jun (c-Jun, JunB, JunD) families. After dimerization, they bind to TPA-responsive elements in the promoter and enhancer region of target genes that have been shown to be important mediators in oncogenic transformation. Likewise, Rel / NF-κB, a widely distributed transcription factor, is associated with many physiological processes including inflammation, cellular proliferation and cancer. NF-κB also plays a key role in the regulation of many genes that are involved in cell proliferation, differentiation, inflammation, cell survival, cell death, angiogenesis, etc. [57].

Topical applications of DMBA to hamster buccal pouch (HBP) increased the expression of NF-κB when compared to vehicle-treated animals. Although dietary BTPs alone did not alter the basal levels, it significantly decreased the DMBA-mediated increase in NF-κB protein expression [58]. Furthermore, pre-treatment with standardized black tea extract inhibited the UV-B-induced c-fos and p53 expression in mouse skin [59]. Pre-treatment with PBPs 1-3, PBP-mix and EGCG significantly decreased the TPA-induced AP-1 and NF-κB DNA binding activities in mouse skin as compared to only TPA treatment, whereas pre-treatments with PBP-4 and PBP-5 did not show any significant difference. Similarly, significant increase was observed in the nuclear protein levels of c-jun, c-fos and NF-κB p65 in DMBA-initiated and TPA-promoted mice during skin tumor development, while significant decrease in these parameters has been demonstrated upon PBPs pre-treatment [56]. Sequential over expression of H-ras gene has been demonstrated in B(a)P-induced lung tumors as compared to control. Administration of TFs (0.02 mg) resulted in differential modulation of the expression of p53 and its associated genes along with H-ras, c-myc at different time points [60].

Oral administration of TF-3 (5 mg / kg) significantly decreased trinitrobenzene sulfonic acid-induced NF-κB activation and degree of colitis. The observed down regulation of NF-κB appears to be due to inhibition of degradation of IκBα by TF-3 [61]. BTE rich in TFs or EGCG (0.02 mg) significantly reduced the B(a)P-induced c-myc gene expression during pulmonary hyperplasia and dysplasia while TFs rich BTE or EGCG had no effect on the expression of H-ras gene during lung carcinogenesis [62]. In a recent study pre-treatment with EGCG (20 or 50 mg / kg BW) has been demonstrated to inhibit topical TPA-induced activation of NF-κB and cyclic AMP response element binding protein in mouse skin that may be mediated *via* suppression of p38 MAPK activation [63]. Overall results suggested that BTPs decreased the DMBA-induced activation of ras protein product p21 and transcription factors AP-1 and NF-κB during HBP tumorigenesis. Together, these studies suggest that BTPs decreased the TPA-induced activation of cellular kinases (ERK, p-38) and transcription factors (AP-1, NF-κB), that modulate the cellular pathways associated with cell proliferation, apoptosis, inflammation, differentiation in TPA-induced tumor promotion events.

c. Effects on Cellular Response Markers

Cell Proliferation

In normal condition, cell proliferation is regulated by proliferation signals, however in transformed cells they are over ridden to cause hyper proliferation under the influence of certain promotion signals. Promotion can be initiated by mitogenic stimuli like growth factors, oxidative stress, hormones etc.

Oral administration of BTE (1-2%) has been shown to decrease cell proliferation during AOM-induced colon carcinogenesis [51]. Dietary administration of BTPs significantly reduced DMBA-induced proliferating cell nuclear antigen (PCNA) labeling index in HBP and DMBA-induced mammary tumors in rats when compared to respective carcinogen controls [28, 58, 64]. Administration of BTE to mice bearing DMBA-induced tumors and in mice bearing UV-B-induced tumors resulted in decrease in the volume of tumors and also showed decreased bromodeoxyuridine (BrdUr) labeling index in squamous cell papillomas, keratoacanthomas and squamous cell carcinomas. These results indicate that oral administration of BTE to tumor bearing mice inhibited cell proliferation and mitotic index [65]. Similarly, BTE (0.05%) significantly decreased cell proliferation in mammary tumors as judged by PCNA labeling indices in transgenic mice [50]. PBPE (1.5%) has been shown to decrease DMH-induced PCNA labeling indices and cyclin D1 protein levels in mice, while PBPE alone had no effect [40]. Pre-treatments with PBPs 1-3 significantly decreased the TPA-induced PCNA labeling indices and the levels of cyclin D1 in mouse skin [56]. Prolong treatment with TFs reduced the B(a)P-induced lung carcinogenesis by differential modulation of p53, H-ras, c-myc and cyclin D1 protein expression at different time points [60]. Administration of TFs significantly decreased B(a)P or NNK-induced proliferation index in mouse lungs [66, 67]. Treatments with TFs rich BTE (0.02 mg) and EGCG (0.01 mg) decreased the B(a)P-induced incidence of hyperplasia, dysplasia and carcinoma in situ in mouse lung at different time points although the extent of decrease was different [68].

BTPs have been shown to inhibit TPA-induced activity of ornithine decarboxylase (ODC) as well as levels of m-RNA in mouse epidermis [69]. Decrease in TPA-induced ODC activity as well as protein and m-RNA levels has also been observed in TF-3 pre-treated (0.5-2.5 μmol) mouse skin [70]. Pre-treatments with PBPs have been shown to reduce TPA-induced ODC activity in mouse epidermis [56]. BTPs modulate the DMBA-induced aberrant expressions of cytokeratins known to be associated with cell proliferation / differentiation [58].

Inflammation

Chronic inflammation is closely linked to tumor promotion and substances with anti-inflammatory activities are anticipated to exert chemopreventive effects particularly during the promotion stage. COX-2 and nitric oxide synthase are important enzymes that mediate inflammatory processes and are known to be up-regulated during tumorigenesis.

Topical application of BTPs (3, 6 mg) has been shown to inhibit TPA-induced interleukin-1 alpha, activity of cyclooxygenase and epidermal inflammation in mouse skin [69]. Furthermore, dietary administration of BTPs (50 mg / kg BW) modulated the AOM-induced COX-2 and iNOS gene expression in tumors [37]. Recent observations have shown that pre-treatments with PBPs 1-3 significantly decreased the TPA-induced COX-2 expression and NF-κB activation in mouse skin [56]. Aqueous suspension of black tea (TFs-2%) has also been shown to reduce AOM-induced ACFs, protein expression of COX-2 and iNOS pathways in rat colon [71]. Topical application of equimolar concentrations of black tea constituents (i.e. TFs) strongly inhibited TPA-induced mouse ear edema and inhibited persistent inflammation as well as interleukin 1β and interleukin-6 protein levels. Pre-treatment with TFs also inhibited the arachidonic acid metabolism *via* both COX and lipooxygenase pathways [72]. Similarly, BTE rich in TFs (0.02 mg) and EGCG (0.01 mg) has been shown to inhibit B(a)P-induced COX-2 expression in mouse lungs [67]. Overall, BTPs decreased carcinogen / promoter-induced inflammation and oxidative stress which have been closely linked to promotion phase of carcinogenesis.

Apoptosis

Apoptosis is characterized by cell shrinkage, membrane blebbing, chromatin condensation and DNA fragmentation. Induction of apoptosis or cell cycle arrest by elimination of cells under stress or genetically damaged cells may represent a protective mechanism by which chemopreventive agent can inhibit promotion / progression stages of carcinogenesis [3]. Tea polyphenols have been shown to induce apoptosis in many cells *in vitro*; however, information on its effect on malignant and non malignant cells in animal models *in vivo* is limited.

Oral administration of BTE (1-2%) to rats treated with AOM during initiation and post-initiation significantly increased apoptotic index in colonic tumors as compared to those in the tumors from the control group [51]. Similarly, it has been shown that orally administered BTE enhanced apoptosis both in nonmalignant and malignant skin tumors induced by UV-B [65]. Dietary administration of BTPs decreased the DMBA-induced Bcl-2 / Bax ratio, p53, Fas and caspase-3 expressions as well as activity of caspase-3, while polyphenon-B alone did not alter these parameters [58]. Dietary administration of BTPs (0.05%) resulted in significant decrease in DMBA-induced expression of Bcl-2 and increase in expressions of Bax, cytochrome C, caspase-3 and caspase-9 in HBP carcinomas [73]. Administration of BTE to transgenic mice resulted in an increase in apoptosis in mammary tissue as reflected by increased levels of cleaved caspase-3 [50]. Similarly, oral administration of BTE significantly increased apoptotic index in AOM-induced colorectal tumors when compared to tumors in carcinogen controls [74]. In another study, pre-treatments with PBPs 1-3 have been shown to abrogate the TPA-induced expression of Bcl-2 and increased the expression of Bax in mouse skin. PBPs-mediated inhibition of TPA-induced anti-apoptotic response was also reflected in increased Bax / Bcl-2 ratio [56]. Treatments with TFs (0.02 mg) and EGCG (0.01 mg) have been shown to increase B(a)P-induced apoptotic index in mouse

Table 3. ***In Vivo*** **Anti-Progressor and Anti-Immunosuppressive Activity of Black Tea Extract / Polyphenols in Rodent Models Bearing Transplanted Tumors / Xenografts**

S. No.	Animal Model	Organ	Carcinogen or tumor cells Dose / Route / Duration	Black tea extract / polyphenols Dose / Route / Duration	Salient observations	Ref.
1	Swiss mice	Liver	EAC $1x10^6$, i.p.	BTE i) 2.5% (w/v), sole drinking fluid, 7 days prior till end ii) 0-5% (w/v), sole drinking fluid, 21 days	i) Protected against EAC-induced hepatotoxicity ii) Decreased tumor cell count in dose-dependent manner, induced apoptosis	[90] [91]
2	Swiss mice	Spleen	EAC i) $1x10^6$, i.p. ii) $1x10^5$, i.p.	i) BTE 2, 3.75, 5% (w/v), sole drinking fluid, 7 days prior to tumor inoculation till end ii) BT 2.5% (w/v), sole drinking fluid, 1 day after tumor inoculation till end, 21 days	i) Inhibited EAC-induced immunosuppression, increased Bcl-2/Bax in immunocytes ii) Reduced TGF-β , IL-10 in tumor cells, initiated Th1/Tc 1 response	[92] [93]
3	Swiss mice	Thymus	EAC i) $1x10^6$, i.p. ii) $1x10^5$, i.p.	BT 2.5% (w/v), sole drinking fluid, 1 day after tumor inoculation till end, 3 wks	i) Prolonged survival of tumor bearing mice, protected thymocytes ii) Protected against tumor-induced thymic involution	[94] [78]
4	SD rats	Liver	DAB 0.06%, diet, 12 wks	Polyphenon-B 0.05%, diet, 24 wks	Decreased incidence of hepatomas, modulated markers of invasion, angiogenesis, HDAC1	[95]
5	Wistar rats	Stomach	MNNG 150 mg/kg BW, i.g. , 3x/2wk-26 wks	Polyphenon-B 0.05%, diet, 1 day after MNNG treatment till end	Suppressed gastric carcinogenesis, Inhibited cell proliferation, infiltration, angiogenesis	[96]
6	Syrian golden hamsters	Buccal pouch	DMBA 0.5%, topical, 3x/wk-14wks	Polyphenon-B, BTF-35 0.05%, diet, 18 wks	i) Reduced tumor volume, cell proliferation, angiogenesis, induced apoptosis in tumors ii) Inhibited HBP carcinogenesis, tumor angiogenesis, down-regulated MMP	[73] [97]
7	SD rats	Mammary gland	DMBA 20 mg/rat, i.g. , 12 wks	Polyphenon-B 0.05%, diet, 4 wks prior till end	Decreased incidence of mammary tumors, modulated XMEs, oxidant, antioxidant status, inhibited cell proliferation, induced cell apoptosis	[28]
8	Athymic (nu/nu) nude mice	Prostate gland	Androgen-sensitive human CaP CWR22Rn1, $1x10^6$, s.c.	BTE 1.25% (w/v), sole drinking fluid, from day 1, 3x/wk-8wks TFs, EGCG 1mg/day/mouse, i.p., from day 1, 54 days	Inhibited growth of tumors, decreased serum PSA, induced apoptosis, decreased VEGF	[98]

BT = Black tea; BTE = Black tea extract; BTF-35 = Black tea extract enriched with theaflavins and catechins; BW = Body weight; CaP = Cancer of prostate gland; DAB = ρ-Dimethylaminoazobenzene; DMBA = 7, 12-dimethylbenz(a)anthracene; EAC = Ehrlich ascites carcinoma; EGCG = (-)-Epigallocatechin-3-gallate; HBP = Hamster buccal pouch; HDAC-1 = Histone deacetylase 1; i.g. = Intragastric; i.p. = Intraperitoneal; IL = Interleukin; MNNG = N-methyl-N'-nitro-N-nitrosoguanidine; MMP = Matrix metalloproteinase; Polyphenon-B = mixture of different catechins, caffeine, oligomers and polymers of tea polyphenols; PSA = Prostate specific antigen; s.c. = Subcutaneous; Tc1 = T cytotoxic cells type 1; Th1 = T helper cell type 2; TFs = Theaflavins; TGF-β = Transforming growth factor beta; VEGF = Vascular endothelial growth factor; XMEs = Xenobiotic metabolizing enzymes.

lungs [75]. This observation is complimented and confirmed by measuring increase in apoptotic markers such as caspase-3 and caspase-7 [67]. TFs have also been shown to modulate the cell proliferative index and apoptotic regulatory gene expression. It has been shown that TFs modulate the p53 dependent apoptotic pathway along with its associated genes such as bax and bcl-2 in B(a)P-induced lung carcinogenesis [60]. BTE rich in TFs or EGCG significantly reduced the B(a)P-induced gene expressions of p53 and bcl-2 and increased the apoptotic index during lung carcinogenesis [62]. Modulation of UV-induced p53 protein expression and apoptotic sunburn cells in mouse skin has also been observed with caffeine [76]. Bax / Bcl-2 ratio has been documented to be an important factor determining the fate of the cell towards death or survival. Taken together, the observations suggest that chemopreventive interventions offered during

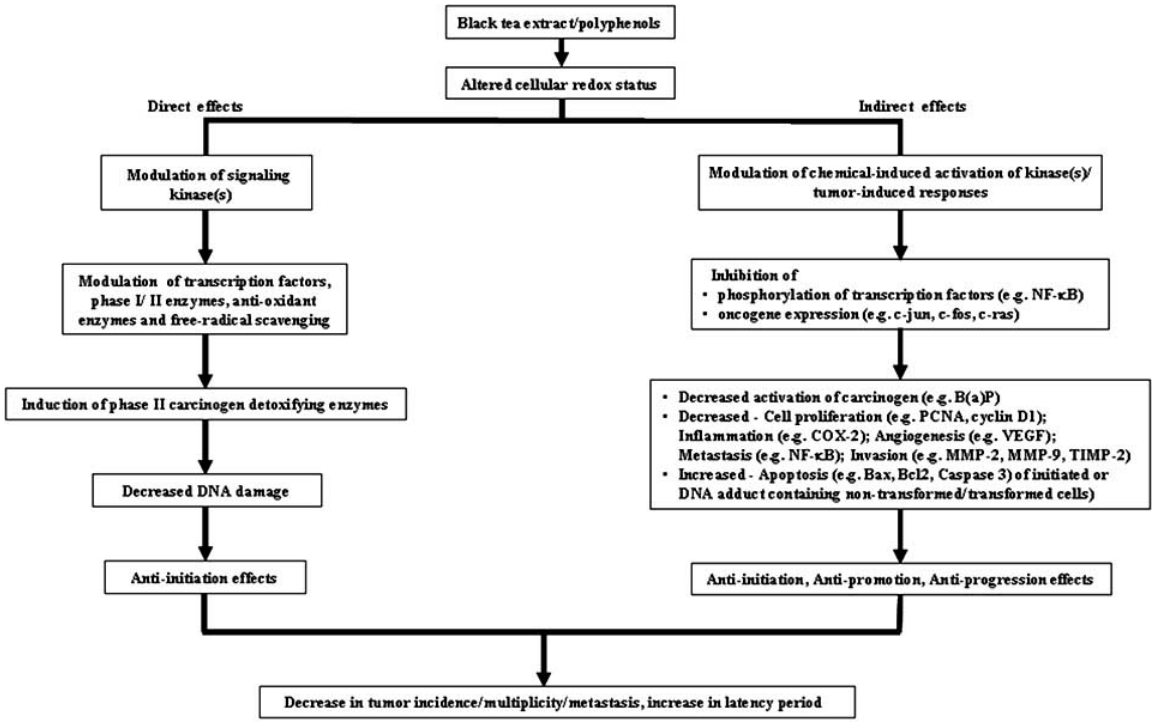

Fig. (2). Summary of *in vivo* chemopreventive effects of black tea extract / polyphenols and underlying mechanisms.

early stage is likely to be effective in decreasing carcinogen-induced cell proliferation while augmenting the probability of initiated cell getting apoptosized. Evaluation of some of the above referred biomarkers is likely to be helpful in monitoring clinical trials with BTPs and evaluating the drug effect measurements.

MECHANISM(S) OF ANTI-PROGRESSOR ACTIVITY

BTPs have been shown to inhibit the growth of transplantable tumors in different animal models and induce apoptogenic signals. Experimental studies have shown that BTPs possess anti-angiogenic, anti-invasive and anti-metastatic effects *in vivo* which have been summarized in Table **3**. Tumor growth requires a continuous and supplementary supply of nutrients and oxygen. To ensure supply of these nutrients, the tumors create new blood vessels. Angiogenesis, the growth of new capillary blood vessels, is crucial for tumor growth and expansion. BTPs have been shown to affect angiogenesis by induction of apoptosis (Bax, Bcl-2), down regulation of cell proliferation (PCNA), pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and its receptor (VEGFR1) (see references in Table **3**). All these observations *in vivo* suggest anti-angiogenic activity of BTPs.

Metastasis is the process by which cancer cells migrate from the tissue of origin to distant sites to form new malignant lesions in other organs. A group of proteolytic enzymes, namely matrix metalloproteinases (MMPs), play a key role in cancer invasion and metastasis. *In vivo* studies demonstrated that carcinogen influences the activity of MMPs, both directly and indirectly. BTPs inhibit the metastasis of tumor cells *in vivo* and the possible mechanism is through the inhibition of MMPs. As shown in Table **3**, BTPs modulate the expression of anti-metatstatic proteins (MMP-2, MMP-9) and the tissue inhibitor of metalloproteases-2 (TIMP-2) (see references in Table **3**). Similarly, inhibition of metallo and serine proteases involved in tumor invasion has also been reported for catechins derived from green tea [77].

Certain cancer cells may secrete immunosuppressive factors to modify the host immune responses. It has been reported that oral administration of black tea significantly reduced depletion of CD4+ and CD8+ cells in peripheral blood. Black tea treatment to tumor bearers inhibited tumor-induced thymic apoptosis and ensured proper functioning of this organ by preventing IL-7 receptor alpha down regulation and restoration of the JAK-STAT cascade. All this information leads to conclusion that black tea protects against tumor-induced immunosupression and thymocyte apoptosis [78].

SUMMARY AND CONCLUSIONS

Chemopreventive efficacy of BTE / BTPs during different stages of carcinogen-induced tumorigenicity has been demonstrated in several experimental models (Tables **2**, **3**). The mechanisms implicated in the inhibition of carcinogenesis by BTPs (Figs. **1**, **2**) involve modulation of signaling kinases or xenobiotic-induced activation / translocation of kinases or modulation of tumor-induced responses ultimately leading to effects on multiple signaling pathways and genes. Considering limited progress, further studies on (i) the characterization of major BTPs, (ii) comparative evaluations of their chemopreventive efficacy, bioavailability and pharmacokinetics and (iii) the mechanisms of chemopreventive actions of BTE and BTE-derived monomeric, oligomeric and polymeric polyphenols are needed before undertaking evaluation of their health effects in human. Information on above referred aspects of major BTPs could provide sound

background for examining the effects of black tea on human health.

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