Antioxidative potential of black tea polyphenols in vitro and protective effects in vivo on mitochondrial redox status during experimental oral carcinogenesis

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Abstract

Introduction: To evaluate the in vitro and in vivo antioxidant properties of the black tea polyphenols Polyphenon-B and BTF-35.

Material and methods: The in vitro antioxidant activity of black tea polyphenols was screened using a panel of assays including 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), hydroxyl radical anion ($OH^{\bullet}$), superoxide anion ($O_2^{\bullet -}$), and nitric oxide (NO) radical scavenging assays as well as assay for reducing power. The in vivo antioxidant potential was evaluated in the 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis model. Hamsters were divided into 6 groups. Animals in groups 1 to 3 were painted with 0.5% DMBA three times a week for 14 weeks. While hamsters in group 1 received no further treatment, animals in groups 2 and 3 received a diet containing 0.05% Polyphenon-B and BTF-35 respectively, from four weeks before DMBA painting until the end of the experiment. Animals in groups 4 and 5 were given Polyphenon-B and BTF-35 alone respectively, and group 6 animals served as controls. All the animals were sacrificed after 18 weeks.

Results: In in vitro studies, both Polyphenon-B and BTF-35 showed high radical scavenging activity and reductive potential. Dietary administration of Polyphenon-B and BTF-35 suppressed DMBA-induced HBP tumours by modulating mitochondrial lipid and protein oxidation and enhancing manganese superoxide dismutase (MnSOD), catalase (CAT), reduced glutathione (GSH) and GSH-dependent enzymes in the buccal pouch and liver.

Conclusions: Our study suggests that the antioxidative properties of tea polyphenols may be responsible for chemoprevention of HBP carcinogenesis. Of the two tea polyphenols analysed, BTF-35 was more effective than Polyphenon-B both in vitro and in vivo.

Key words: antioxidants, chemoprevention, mitochondria.

Introduction

Oxidative stress arising due to overproduction of reactive oxygen species (ROS) coupled with deficiency of antioxidant defence mechanisms has been implicated in the pathogenesis of cancer, the second most common cause of death worldwide [1]. Mitochondria, a rich source of ROS production via the electron transport chain, is an ideal organelle to analyze oxidative stress and

carcinogenesis [2]. Under normal physiological conditions, the highly toxic ROS are quenched by the mitochondrial antioxidant defence systems. In particular, mitochondrial catalase (CAT), manganese superoxide dismutase (MnSOD), as well as glutathione (GSH), in conjunction with glutathione peroxidase (GPx) and glutathione S-transferase (GST), regulate inner mitochondrial membrane permeability by detoxifying ROS produced during electron transport and confer protection against lipid peroxidative damage [3]. However, accumulating evidence suggests that ROS generated by mitochondria during neoplastic transformation act as second messengers in intracellular signalling cascades that induce and maintain the oncogenic phenotype [1, 4]. Scavengers of ROS have therefore evolved as effective chemopreventive agents. Several diet-derived agents that upregulate mitochondrial antioxidant defences have assumed importance in cancer chemoprevention in recent years [5, 6].

There is strong and consistent evidence to demonstrate that a diet high in fruits, vegetables and beverages is associated with decreased risk of many cancers [7]. The potential health benefits of dietary agents have been attributed to the effects of specific ingredients/phytochemicals such as vitamins, dietary fibre, carotenoids, terpenes, alkaloids, isoflavones, indoles and polyphenols [8]. Among the various phytochemicals, polyphenols in tea have attracted the focus of attention because of their wide range of beneficial effects.

Tea made from the leaves of *Camellia sinensis* is one of the most widely consumed beverages next to water. About 3 billion kilograms of tea are produced and consumed annually, of which black tea accounts for nearly 80%. The predominant consumption of black tea has stimulated interest in its possible beneficial effects. The major health benefits of black tea have been ascribed to the presence of polyphenols, such as catechins, thearubigins and theaflavins that are reported to have radical scavenging properties [9, 10]. Although the antioxidant potential of tea polyphenols can be screened by in vitro radical scavenging assays, it is important to demonstrate antioxidant-enhancing effects in vivo in an animal tumour model before embarking on clinical trials. Previously, we reported the effects of black tea polyphenols on cellular redox status in various experimental animal tumour models [11-14]. However, the effect of black tea polyphenols in mitigating mitochondrial oxidative stress during carcinogenesis has not been documented. We therefore undertook the present study to correlate in vitro antioxidant potential of two black tea extracts, Polyphenon-B (a mixture of black tea polyphenols), and BTF-35 (a well characterized black tea extract enriched with theaflavins and catechins) with in vivo modulatory effects on the mitochondrial redox status in the hamster buccal pouch carcinogenesis model. The extent of mitochondrial lipid and protein oxidation and the status of the antioxidants, MnSOD, CAT, GSH, GPx and GST in the buccal pouch, the target organ as well as in host liver were used to biomonitor chemoprevention.

Material and methods

Chemicals

Ascorbic acid, 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), bovine serum albumin, 7,12-dimethylbenz[a]anthracene (DMBA), 2,4-dinitrophenylhydrazine, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), GSH, nicotinamide adenine dinucleotide phosphate reduced (NADPH), potassium persulfate and 2-thiobarbituric acid (TBA) were purchased from Sigma Chemical Company, St. Louis, MO, USA. Polyphenon-B and BTF-35 were kindly provided by Mitsui Norin Co., Ltd., Tokyo, Japan. The composition of Polyphenon-B and BTF-35 are given in Table I. All other reagents used were of analytical grade.

In vitro free radical scavenging assays

1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay

The free radical scavenging capacities of Polyphenon-B and BTF-35 were evaluated by DPPH assay following the methodology described by Blois [15]. The reaction is based on the one-electron reduction of DPPH. In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species, the absorption decreases. Briefly, 0.25 mM solution of DPPH• in methanol was prepared and 1 ml of this solution was added to 1 ml of Polyphenon-B and BTF-35 solution in

Table I. Composition of Polyphenon-B and BTF-35

Polyphenolic constituent	Polyphenon-B (% w/w)	BTF-35 (% w/w)
Epigallocatechin (EGC)	–	0.1
Epicatechin (EC)	0.4	0.2
Epigallocatechin gallate (EGCG)	1.4	2.6
Epicatechin gallate (ECG)	0.1	2.1
Gallocatechin gallate (GCG)	0.2	0.3
Catechin gallate (CG)	–	0.1
Catechin (C)	–	0.1
Free theaflavin	0.32	7.1
Theaflavin monogallate-A	0.14	8.3
Theaflavin monogallate-B	0.15	2.6
Theaflavin digallate	0.24	9.8
Tannin	35.6	–
Caffeine	4.9	0.5

methanol at different concentrations (1-30 µg/ml). After 20 minutes, the absorbance was measured at 517 nm. Ascorbic acid was used as a positive control. The percentage DPPH decolourisation of the sample was calculated by the equation,
% DPPH scavenging = $[(A_{control} - A_{extract})/A_{control}] \times 100$, where A is the absorbance.

2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) assay

The total antioxidant potential was measured by ABTS assay as described by Miller et al. [16]. This assay measures the relative ability of antioxidant substances to scavenge the $ABTS^{\bullet+}$ cation radical generated in the aqueous phase. The 3.5 ml reaction mixture contained 0.17 mM ABTS, 1-10 µg Polyphenon-B or BTF-35, and phosphate buffer (pH 7.4). The absorbance was measured at 734 nm.

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity was determined by the method of Halliwell et al. [17]. The method detects hydroxyl radicals produced by the reduction of H_2O_2 by iron, in the presence of ascorbic acid, based on the ability to degrade deoxyribose to form products which on heating with TBA form a pink coloured chromogen. Polyphenon-B and BTF-35 when added to the reaction mixture compete with deoxyribose for hydroxyl radicals, diminishing colour development. Briefly, the reaction mixture, in a final volume of 1.0 ml, containing 0.4 ml of 20 mM sodium phosphate buffer (pH 7.4), 0.1 ml of 1-10 µg/ml of Polyphenon-B/BTF-35, 0.1 ml of 60 mM deoxyribose, 0.1 ml of 10 mM H_2O_2, 0.1 ml of 1 mM ferric chloride, 0.1 ml of 1 mM EDTA and 0.1 ml of 2 mM ascorbic acid, was incubated at 37°C for 1 h. The reaction was terminated by adding 1 ml of 17 mM TBA and 1 ml of 17 mM trichloroacetic acid (TCA). The mixture was then boiled for 15 min, cooled in ice, and the absorbance measured at 532 nm. Ascorbic acid was used as a positive control. Distilled water in place of Polyphenon-B and BTF-35 or ascorbic acid was used as a control and the sample solution without adding deoxyribose as a sample blank.

Superoxide anion scavenging activity

The superoxide anion scavenging activity of Polyphenon-B and BTF-35 was determined by the method of Nishimiki et al. [18]. Superoxide anion derived from dissolved oxygen by a PMS/NADH coupling reaction reduces nitroblue tetrazolium (NBT), which forms a violet coloured complex. A decrease in colour after addition of the antioxidant is a measure of its superoxide scavenging activity. The reaction mixture in a total volume of 3 ml contained phosphate buffer (100 mM, pH 7.4), NBT (1 mM) solution, NADH (1 mM) and sample solution of Polyphenon-B/BTF-35 (1-10 µg/ml) in methanol. The reaction was started by adding 1 ml of PMS solution (1 mM) to the mixture. The reaction mixture was incubated at 25°C for 5 min, and the absorbance at 560 nm was measured against a blank. Ascorbic acid was used as a positive control, and distilled water in place of samples or ascorbic acid as the blank.

Nitric oxide radical inhibition assay

The nitric oxide radical inhibition activity of Polyphenon-B and BTF-35 was measured by the method of Garratt [19]. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Briefly, sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with different concentrations of Polyphenon-B/BTF-35 dissolved in methanol and incubated at room temperature for 150 min. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride) was added. The reaction mixture without Polyphenon-B and BTF-35 served as the control. The absorbance of the chromophore formed was read at 546 nm.

Reducing power

The reductive potential of Polyphenon-B and BTF-35 was determined according to the method of Oyaizu [20] based on the chemical reaction of Fe(III) to Fe(II). Different concentrations of Polyphenon-B and BTF-35 and ascorbic acid standard (1-10 µg/ml) in 1 ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1% w/v). The mixture was incubated at 50°C for 20 min, followed by addition of 2.5 ml of TCA (10% w/v). The mixture was centrifuged for 10 min at 1000 g, the upper layer (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1% w/v), and the absorbance was measured at 700 nm in a spectrophotometer. A higher absorbance indicates greater reducing power.

Animals and diet

The experiment was carried out with male Syrian hamsters aged 6-10 weeks weighing between 90-110 g obtained from the Central Animal House, Annamalai University, India. The animals were housed six to a polypropylene cage and provided food and water *ad libitum*. The animals were maintained in a controlled environment under standard conditions of temperature and humidity with an alternating 12 hours light/dark cycle. The animals were maintained in accordance with the guidelines of the

Indian Council of Medical Research and approved by the ethical committee, Annamalai University. The experimental diet was prepared every day by mixing Polyphenon-B and BTF-35 to a pre-weighed standard pellet diet (Mysore Snack Feed, Mysore, India). The diet was replenished every day and the food consumption was recorded.

Treatment schedule

The hamsters were randomized into experimental and control groups and divided into 6 groups of 6 animals each. At 10 weeks of age, the hamsters in groups 1 to 3 were painted with a 0.5% solution of DMBA in liquid paraffin on the right buccal pouches using a number 4 brush three times a week for 14 weeks. Each application leaves approximately 0.4 mg of DMBA [21]. Hamsters in group 1 received no further treatment. Animals in groups 2 and 3 received a diet containing Polyphenon-B and BTF-35 respectively from four weeks before DMBA painting when they were 6 weeks of age and continued until the final exposure to carcinogen. Animals in groups 4 and 5 were given Polyphenon-B and BTF-35 alone respectively as in groups 2 and 3. Group 6 animals received the basal diet and served as controls. The dose for black tea polyphenols used in the present study corresponds to the daily intake of four cups of tea (30-40 mg of tea polyphenols per kilogram body weight by humans) [22]. The experiment was terminated at the end of 18 weeks and all animals were sacrificed by cervical dislocation after an overnight fast. Before an animal was killed, the right buccal pouch was grossly inspected to evaluate premalignant lesions or tumour development and photographed. The tumour burden was calculated by multiplying the mean tumour volume ($4/3\ \pi r^3$) (r=1/2 tumour diameter in mm) with the mean number of tumours. Fresh tissues were used for estimations. Biochemical estimations were carried out in the buccal pouch and liver mitochondrial fractions of experimental and control animals.

Isolation of mitochondria

The pouch and liver mitochondria were isolated as described by the method of Johnson and Lardy [23]. Immediately after sacrifice, the pouch and liver tissues were removed, washed twice with ice-cold buffer (sucrose 0.25 M, Tris HCl 5 mM pH 7.4 and EDTA 1 mM) and minced into small sections with scissors. The homogenate was prepared by using 10 ml of cold buffer and centrifuged at 1000 g for 10 min at 0-4°C. All the subsequent steps were done at 0-4°C. The pellet containing the nucleus, RBC and cell debris was discarded and the supernatant was spun at 9300 g for 7 minutes. The pellet contains three layers. The middle layer, which is medium to dark brown in colour, containing largely intact mitochondria and microsomes, was carefully removed and resuspended in 10 ml of cold buffer and again centrifuged at 9300 g for 10 min. The final pellet was suspended in 1.15% KCl, gently homogenized and used for biochemical assays.

Biochemical assays

Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was assayed in tissue mitochondrial fractions by the method described by Ohkawa et al. [24]. Protein oxidation was measured by the method of Levine et al. [25] based on the reaction of the carbonyl group with 2,4-dinitro-phenylhydrazine. Mn-SOD activity was assayed as described by Oberley and Spitz [26] based on the half-maximal inhibition of NBT reduction. The activity of catalase was assayed by the method of Sinha [27] based on the utilization of hydrogen peroxide by the enzyme. GSH was determined by the method of Anderson [28] based on the development of a yellow colour when DTNB is added to compounds containing sulfhydryl groups. GPx activity was assayed by the method of Rotruck et al. [29] with modifications. A known amount of enzyme preparation was incubated with hydrogen peroxide in the presence of GSH for 10 min. The amount of hydrogen peroxide utilized was determined by estimating GSH content by the method of Anderson [28]. The activity of GST was determined as described by the method of Habig et al. [30] by following the increase in absorbance at 340 nm using CDNB as substrate. The protein content was estimated by the method of Lowry et al. [31].

Statistical analysis

The data are expressed as mean ± standard deviation (SD). Tumour incidence was statistically compared using χ^2-test combined with Yates' correction. Statistical analysis on the data for tumour burden and reducing power was carried out using Students' *t*-test. The data for biochemical assays were analysed using analysis of variance (ANOVA) and the group means were compared by the least significant difference test (LSD). The results were considered statistically significant if the p value was <0.05. IC_{50} values were determined by plotting dose response curves of radical scavenging activities vs concentration of black tea polyphenols using GraphPad Prism version 4.00 for Windows (GraphPadTM Software Inc., San Diego, California, USA).

Results

Figure 1 and Table II show the free radical scavenging effect of ascorbate, Polyphenon-B and BTF-35 and their IC_{50} values. Both Polyphenon-B and BTF-35 showed concentration-dependent antiradical activity resulting from reduction of $DPPH^{\bullet}$, $ABTS^{\bullet+}$, superoxide ($O_2^{\bullet-}$), hydroxyl ($OH^{\bullet}$)

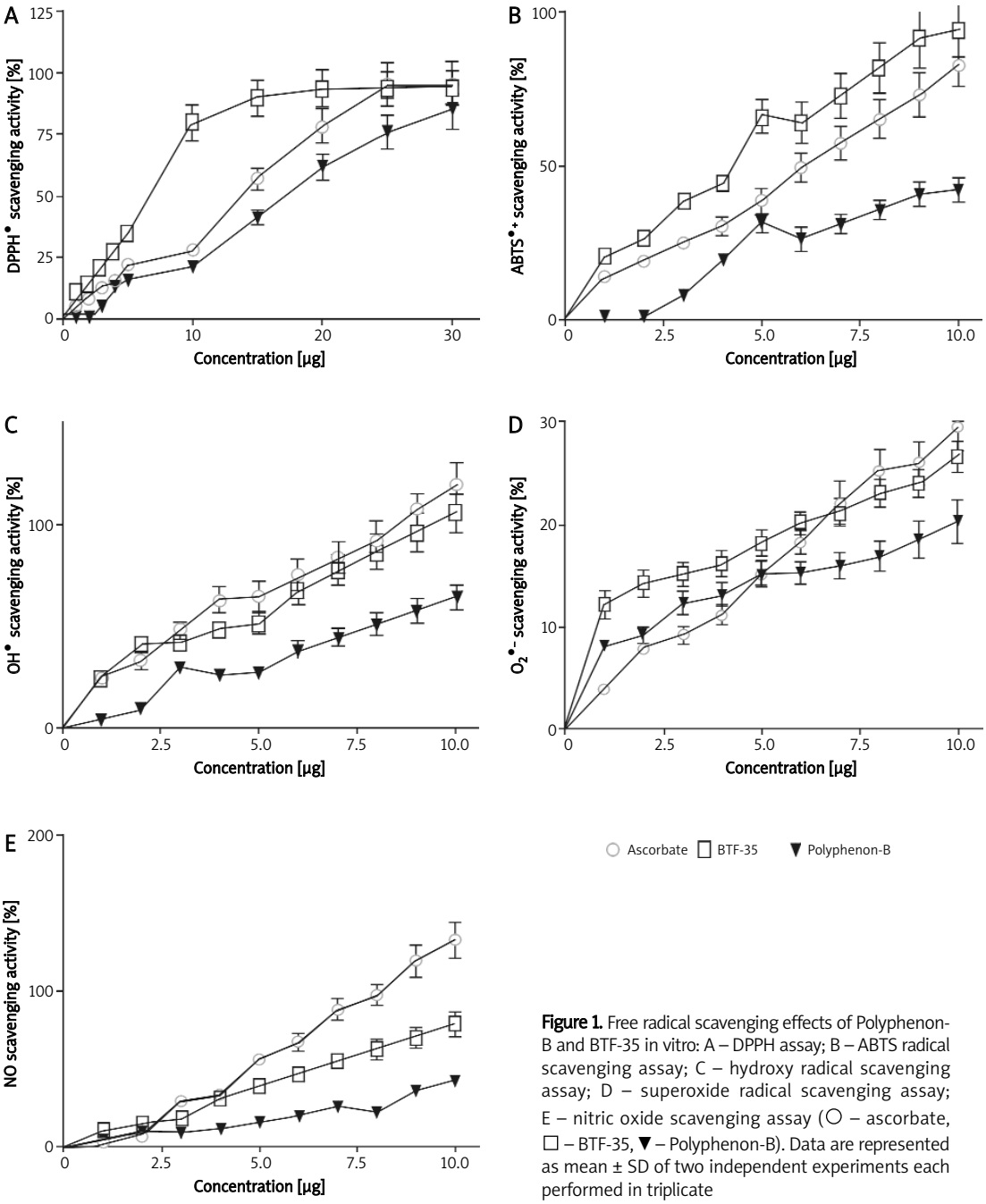


Figure 1. Free radical scavenging effects of Polyphenon-B and BTF-35 in vitro: A – DPPH assay; B – ABTS radical scavenging assay; C – hydroxy radical scavenging assay; D – superoxide radical scavenging assay; E – nitric oxide scavenging assay (○ – ascorbate, □ – BTF-35, ▼ – Polyphenon-B). Data are represented as mean ± SD of two independent experiments each performed in triplicate

and nitric oxide (NO) radicals to non-radical form. BTF-35 was more effective in scavenging DPPH, ABTS and superoxide radicals compared to the positive control, ascorbic acid. The order of DPPH, ABTS and superoxide radical scavenging effect was BTF-35 > ascorbic acid > Polyphenon-B, whereas the order of OH• and nitric oxide radicals scavenging activity was ascorbic acid > BTF-35 > Polyphenon-B. The reducing power of ascorbic acid, Polyphenon-B and BTF-35 increased gradually with increasing concentration (Figure 2) and the order of reduction potential was ascorbic acid > BTF-35 > Polyphenon-B. We found a significant association between the polyphenolic content of black tea extracts and the antioxidative potential. Of the two black tea extracts analysed, BTF-35, enriched in catechins and theaflavins, showed more radical scavenging activity as well as reductive potential than Polyphenon-B.

Table III shows the mean body weight, tumour incidence, and tumour burden in experimental animals. Hamsters in group 1 showed a tendency to be lower in body weight gain during the experiment and the mean final body weights were significantly decreased compared to controls (group 6). Treatment with Polyphenon-B and BTF-35

Table II. IC_{50} values of ascorbate and black tea polyphenols against various free radicals

Antioxidant activity	IC_{50} values		
	Ascorbate	Polyphenon-B	BTF
DPPH scavenging (μg/ml)	15.73	17.42	10.31
ABTS scavenging (μg/ml)	6.07	10.90	4.50
$OH^{\bullet}$ scavenging (μg/ml)	3.63	7.77	4.10
$O_2^{\bullet -}$ scavenging (μg/ml)	50.16	94.88	48.22
NO scavenging (μg/ml)	4.46	13.44	6.42

IC_{50} values were determined by plotting dose response curves of radical scavenging activities vs. concentration of black tea polyphenols using GraphPad Prism version 4.00 for Windows (GraphPadTM Software Inc., San Diego, California, USA)

Table III. Mean body weight, tumour incidence and tumour burden in the buccal pouch of experimental animals (mean ± SD; n=6).

Group	Treatment	Body weight gained (g)	Tumour incidence	Tumour burden[a] mm^3	Squamous cell carcinoma
1.	DMBA	12.76[b]	6/6	127.21±87.42	# (100%)
2.	DMBA + Polyphenon-B	28.82[c]	1/6[d]	8.31±7.92[c]	# (16.6%)[c]
3.	DMBA + BTF-35	26.26[e]	0/6[f]	–	–
4.	Polyphenon-B	37.70	–	–	–
5.	BTF-35	37.91	–	–	–
6.	Control	40.21	–	–	–

a – mean tumour burden was calculated by multiplying the mean tumour volume with the mean number of tumours (tumour volume was calculated using 4/3 πr^3, where r=1/2 tumour diameter in mm)
b – significantly different from group 6 by Student's t-test ($p<0.001$)
c – significantly different from group 1 by Student's t-test ($p<0.05$)
d – significantly different from group 1 by χ^2-test combined with Yates' correction ($p<0.02$)
e – significantly different from group 1 by Student's t-test ($p<0.05$)
f – significantly different from group 1 by χ^2-test combined with Yates' correction ($p<0.004$)

to DMBA-painted animals significantly increased the mean final body weights in group 2 and 3 animals respectively compared to group 1. No significant differences in the body weights were observed in groups 4 to 6. In hamsters painted with DMBA alone (group 1), the tumour incidence was 100%. These tumours were large and exophytic with a mean tumour burden of 127 mm^3. Administration of Polyphenon-B and BTF-35 effectively suppressed the development of HBP carcinomas. One of the 6 animals treated with DMBA and Polyphenon-B developed SCC, while no tumours were observed in animals treated with DMBA and BTF-35. In groups 4-6, the epithelium was normal, intact and continuous.

Figure 3 shows the effect of treatment with Polyphenon-B and BTF-35 on mitochondrial lipid and protein oxidation in the pouch and liver of experimental and control animals. The extent of lipid peroxidation and the formation of protein carbonyl were significantly lower in the pouch and higher in the liver mitochondria of DMBA-treated animals (group 1) compared to controls. Dietary administration of Polyphenon-B (group 2) and BTF-35 (group 3) significantly modulated DMBA-induced changes in lipid and protein oxidation, reflected by a significant increase in the pouch mitochondria with concomitant decrease in the liver mitochondria compared to group 1. Administration of chemopreventive agents alone (groups 4 and 5) significantly reduced the extent of lipid peroxidation and protein oxidation in the pouch and liver mitochondria compared to controls.

The activities of the antioxidant enzymes Mn-SOD, CAT, GPx and GST, and the levels of GSH in the pouch and liver mitochondria of experimental and control animals are presented in Figure 4. In DMBA-treated animals (group 1), the activities of MnSOD and CAT were significantly decreased in the pouch and liver mitochondria, whereas mitochondrial GSH and the activities of GPx and GST were significantly increased in the pouch and decreased in the liver compared to controls (group 6). Dietary administration of Polyphenon-B and BTF-35 significantly increased MnSOD, CAT, GSH and GSH-dependent enzymes in groups 2 and 3 animals respectively compared to group 1. Administration of tea polyphenols alone significantly enhanced all the antioxidants in groups 4 and 5 animals compared to controls.

Discussion

Tea polyphenols have been reported to exert anti-cancer properties both in vitro and in vivo [32, 33].

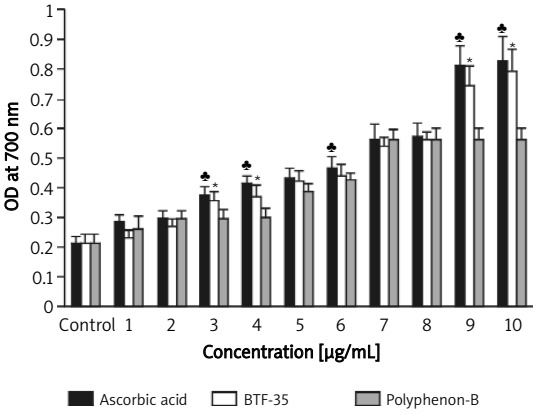
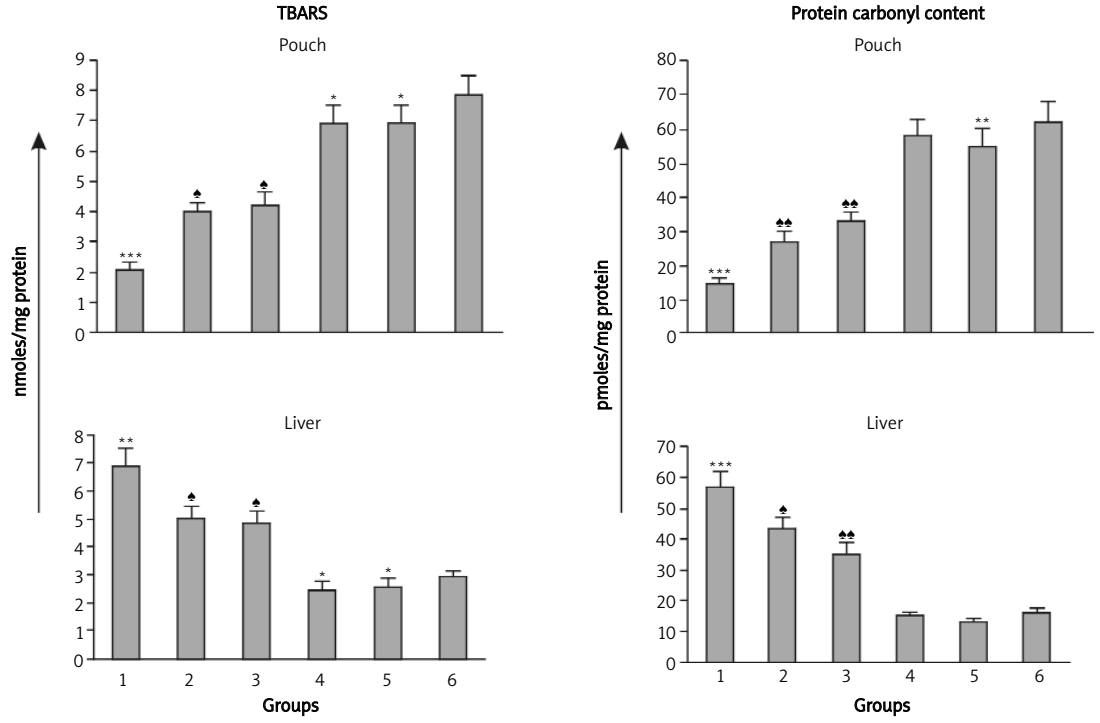


Figure 2. Reducing potential of Polyphenon-B and BTF-35 in vitro: ♣ – significantly different from Polyphenon-B and BTF-35 ($p<0.05$) by Student's *t*-test; * – significantly different from Polyphenon-B ($p<0.05$)

However, epidemiological studies on tea consumption and human cancer prevention still remain inconclusive. Although a transient increase in plasma antioxidant capacity following tea consumption has been observed, the contribution of antioxidants in tea to inhibition of carcinogenesis has not been fully delineated [34]. The results of the present study provide evidence for the antioxidant potential of two black tea polyphenols, Polyphenon-B and BTF-35. The antioxidant potential assessed by DPPH•, $ABTS^{\bullet+}$, $O_2^{\bullet-}$, OH• and NO radical scavenging assays suggest that the phenolic compounds found in black tea are able to react directly with reactive radicals and scavenge them. The greater radical scavenging efficacy of BTF-35 may be attributed to higher concentrations of catechins and theaflavins that are recognised as potent antioxidants.

In the present study, DMBA painted hamsters exhibited reduced mitochondrial lipid and protein oxidation accompanied by enhanced activities of GSH-dependent antioxidants. However, the activities of MnSOD and CAT were significantly decreased in HBP tumours. An increase in the activity of GPx with decreased SOD and CAT has been reported in oral cancer as well as in the hamster cheek pouch carcinoma cell line HCPC-1 [35, 36]. Reduced activities of MnSOD and CAT reported in fast growing tumour tissue can cause accumulation of superoxide ions ($O_2^{\bullet-}$) and H_2O_2, leading to oxidative damage to mitochondrial proteins, lipids and nucleic acids [37]. In particular, hepatic metabolism of DMBA produces highly reactive metabolites and ROS that induce lipid peroxidation or act as second messengers of the primary free radicals that initiate lipid peroxidation



1. DMBA 2. DMBA + Polyphenon-B 3. DMBA + BTF-35 4. Polyphenon-B alone 5. BTF-35 alone 6. Control

Figure 3. Effect of Polyphenon-B and BTF-35 on TBARS and protein carbonyl content in the buccal pouch and liver mitochondria of experimental and control animals (mean ± SD; n=6): * – significantly different from group 6 ($p<0.05$) ANOVA followed by LSD; ** – significantly different from group 6 ($p<0.01$); *** – significantly different from group 6 ($p<0.001$); ♠ – significantly different from group 1 ($p<0.05$) ANOVA followed by LSD; ♠♠ – significantly different from group 1 ($p<0.01$)

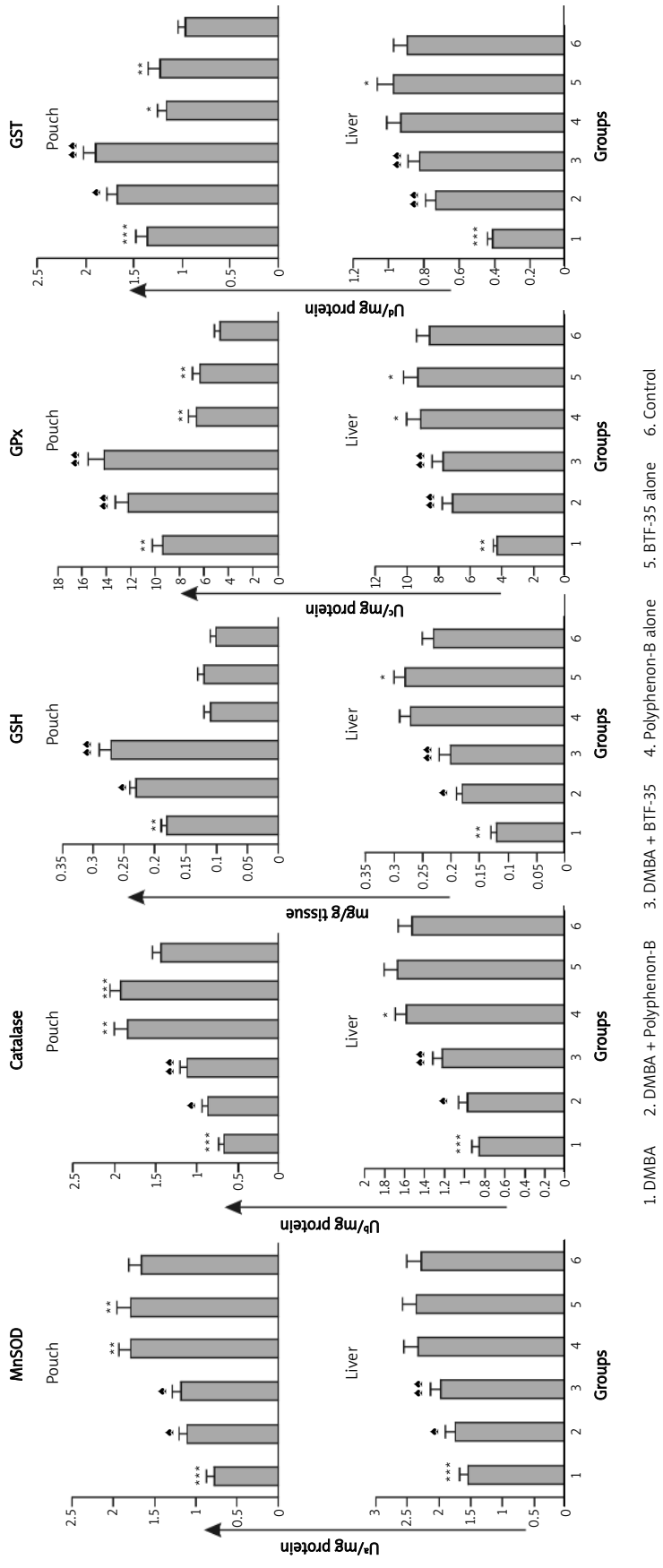


Figure 4. The activities of MnSOD, catalase, GPx and GST and GSH levels in the buccal pouch and liver mitochondria of experimental and control animals (mean ± SD; n=6): * – significantly different from group 6 ($p<0.05$) ANOVA followed by LSD; ** – significantly different from group 6 ($p<0.01$); *** – significantly different from group 6 ($p<0.001$); ♣ – significantly different from group 1 ($p<0.05$) ANOVA followed by LSD; ♣♣ – significantly different from group 1 ($p<0.01$); a – amount of enzyme required to give 50% inhibition of NBT reduction; b – µmoles of H_2O_2 utilised per second; c – µmoles of GSH utilized/min; d – µmoles of CDNB conjugated with GSH/min

[38]. In contrast to HBP tumours, lipid and protein oxidation in the hepatic mitochondria of tumour-bearing animals were enhanced, associated with compromised hepatic mitochondrial antioxidant defences that may be ascribed to increased utilization to scavenge ROS and/or sequestration by the tumours. Thus the tumour and the host liver appear to comprise two separate metabolic compartments with respect to their susceptibility to lipid and protein oxidation.

Dietary administration of Polyphenon-B and BTF-35 significantly suppressed DMBA-induced HBP tumours accompanied by modulation of the mitochondrial oxidant-antioxidant status in the target organ as well as in the host liver. Both the agents reversed the susceptibility to lipid and protein oxidation while simultaneously increasing the antioxidant status in the buccal pouch. The antioxidant enhancing effects of black tea polyphenols in the hamster buccal pouch may be viewed as an '*electrophilic counterattack response*' to DMBA-induced elevation of antioxidants and is potentiated by similar reports in the literature [11, 39]. In contrast to the pouch tissue, both Polyphenon-B and BTF-35 reduced the extent of lipid and protein oxidation in the liver, accompanied by an increase in the antioxidant enzyme activities. Chemopreventive agents are known to upregulate the ability of the liver to metabolise carcinogens and induce antioxidant enzymes, altering tumour development at extrahepatic sites. Thus the differential oxidative events induced in the tumour and host tissues by Polyphenon-B and BTF-35 reflect their antiproliferative effects and upregulation of host antioxidant defences. These findings substantiate the observation of Yamamoto et al. [40] that EGCG, a tea polyphenol, functions as an ROS inducer in tumour cells and as an ROS scavenger in normal cells.

Although antioxidant activities of black tea polyphenols have been demonstrated both in vitro and in vivo, this study is the first to compare the in vitro antioxidant effect of black tea polyphenols and their modulatory effects on mitochondrial oxidant-antioxidant status during HBP carcinogenesis. The modulatory effects of Polyphenon-B and BTF-35 on mitochondrial redox status may be attributed to the antioxidative property of tea polyphenols as evidenced by the radical scavenging and reductive potential. Recently, we reported the influence of black tea polyphenols on mitochondrial redox status during MNNG-induced gastric carcinogenesis [41]. Choudhary and Verma [42] demonstrated that black tea extract ameliorates aflatoxin-induced lipid peroxidation by inducing enzymic and non-enzymic antioxidants. Several studies by us and other workers have also documented a positive correlation between the chemopreventive efficacy of black tea and its enhancing effect on antioxidant defence systems [11-14, 33, 43]. Taken together, these findings strengthen the hypothesis that dietary antioxidants are effective chemopreventive agents.

In conclusions, the results of the present study provide evidence that BTF-35, with greater in vitro antioxidant activity, inhibits HBP carcinomas more effectively than Polyphenon-B by modulating mitochondrial redox status in the tumour and host tissues. The greater efficacy of BTF-35 may be due to the rich polyphenolic content, which can potentially scavenge the free radicals generated and impede their deleterious effects. However, additional studies on the effect of BTF-35 on electron transport chain activity, mitochondrial membrane stability and gene expression are required to validate the chemopreventive effect of BTF-35.

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