Food and Chemical Toxicology 131 (2019) 110551

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

White tea - A cost effective alternative to EGCG in fight against benzo(a) pyrene (BaP) induced lung toxicity in SD rats

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ARTICLE INFO

Keywords:
Oxidative stress
Lung tissue
SD rats
Endogenous oxidants

ABSTRACT

Tea is a natural resource of catechins and exhibits antioxidative and anticancer activities. This study was designed to elucidate the comparative efficacy of white tea and pure EGCG in containing benzo (a) pyrene (BaP)-induced pulmonary stress. Rats were treated with white tea extract (WT) (1%) and pure EGCG at a dose of 80μg/ml in drinking water on alternate days for 12 weeks (4 weeks prior, during and after BaP treatment). BaP(50 mg/kg b. wt) was administered to rats orally in olive oil twice a week for four weeks. The indices such as stress biomarkers (LPO, PCC & ROS), antioxidant enzymes (SOD, CAT, GSH, GST, GR, GPx) activities and lung histoarchitecture were assessed. BaP administration enhanced the levels of inflammatory markers (NO and citrulline) and reduced activities of antioxidant enzymes. We observed similar antioxidant efficacy by both WT and EGCG as seen by their ameliorative action in restoring BaP induced oxidative and inflammatory stress as well as lung histoarchitecture. Our findings suggest that WT is equally beneficial as EGCG in maintaining the integrity of alveoli and is a potential candidate to be used as a cost effective and protective agent in conditions of BaP-induced lung damage.

1. Introduction

Medicinal herbs which are widely used in traditional medicine practices have gained recognition over time as a resource of drug discovery and development. Tea is one of the most consumed beverages worldwide and is being extensively explored for its health benefits (Chen et al., 2011). White tea (WT), which is extracted from *Camellia sinensis,* contains high levels of catechins but is rarely used as a beverage. Epigallocatechin gallate (EGCG), an active component of white tea, has earlier been reported to exhibit anti-carcinogenic, immune-boosting and antioxidative properties that can improve human health (Unachukwu et al., 2010; Zhang et al., 2007; Chhabra et al., 2001; Peschard et al., 2007; Fujiki et al., 2002; Siddiqui et al., 2011).

The enormous health benefits of EGCG have encouraged various pharmaceutical service providers to formulate pure EGCG as a preventive intervention. But, the process of isolation, purification and characterization of EGCG from WT extract is relatively time consuming and also increases the cost of formulation. So, it is worthwhile to elucidate the comparative efficacy of pure EGCG and WT extract to validate the extent of protection exerted by WT extract vis-à-vis equivalent amount of pure EGCG. Thus, the present work was undertaken to explicate the comparative efficacy of WT extract and pure EGGC equivalent to the amount present in WT extract against benzo(a)pyrene (BaP) induced pulmonary toxicity.

We induced pulmonary toxicity in rats by administering BaP (Srivastava et al., 2000), which is discharged in the environment from incomplete combustion of organic compounds. The major contributory factors for BaP involve engine exhaust, cigarette smoke, soil, water and food (Cao et al., 2005). The enzyme dihydrodiol dehydrogenase metabolizes BaP into quinones which in turn undergo redox cycling and induce oxidative stress via reactive oxygen species (ROS) (Penning et al., 1999). The increased level of oxidative stress is associated with elevated levels of oxidative stress biomarkers such as protein carbonyl content (PCC) and lipid peroxidation (LPO) (Poirier, 2004; Sehgal et al., 2013). Elevated oxidative stress biomarkers and decreased level of antioxidants are considered to be pivotal for enhanced susceptibility of cellular macromolecules to oxidative stress (Kasala et al., 2015). The elevated levels of reactive oxygen species are associated with diverse medical conditions as they are linked to various signaling cascades and in particular H_2O_2 is believed to regulate the activities of tyrosine kinases, phosphatases and transcription factors (Chiarugi and Fiaschi, 2007; Rhee et al., 2000; Storz, 2005).

This is the first study of its kind to appraise the comparative efficacy of pure EGCG and white tea in preventing BaP induced pulmonary stress. The study was executed with an emphasis on various indices that included a) *in vitro* determination of free radical scavenging activity of

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https://doi.org/10.1016/j.fct.2019.05.059
Received 5 January 2019; Received in revised form 28 May 2019; Accepted 31 May 2019
Available online 01 June 2019
0278-6915/

Table 1
Experimental design.

S. No.	Groups	Weeks (1–4)	Weeks (5–8)	Weeks (9–12)
Group 1	Normal control (NC)	Drinking water	Drinking water	Drinking water
Group 2	BaP	Drinking water	BaP (50 mg/kg b.wt.)	Drinking water
Group 3	EGCG	EGCG (80 μg/ml)	EGCG (80 μg/ml)	EGCG (80 μg/ml)
Group 4	WT	WT (1%)	WT (1%)	WT (1%)
Group 5	WT + BaP (WB)	WT (1%)	WT (1%) + BaP (50 mg/kg b.wt.)	WT (1%)
Group 6	EGCG + BaP (EB)	EGCG (80 μg/ml)	EGCG (80 μg/ml) + BaP (50 mg/kg b.wt.)	EGCG (80 μg/ml)

pure EGCG and WT extract, b) *in vivo* investigations to assess the effects of EGCG and WT on functional efficacy of endogenous antioxidants and oxidative stress biomarkers c) lung histoarchitecure examination in BaP induced pulmonary stress in rats.

2. Materials and methods

2.1. Chemicals

BaP, Bovine serum albumin, Glutathione reductase, Reduced glutathione, Chloro-2,4-dinitro benzene, 2,4-dinitrophenyl hydrazine, 5,5-dithiobis-2-nitrobenzoic acid were purchased from Sigma Chemical Co. India. EGCG (Cat No. 588500) was purchased from Toronto Research Company, Canada. White tea was procured from Budwhite teas Pvt. Ltd, New Delhi, India. The reagent kits for the analysis of liver marker enzymes were procured from Reckon Diagnostics Pvt. Ltd. All other chemicals used were of analytical grade and procured from reputed Indian manufactures.

2.2. Tea preparation

WT extract was prepared by soaking loose tea leaves (1 g in 100 ml) in hot water and were kept at 85 ± 3 °C for 5 min (Kumar et al., 2012a,b). The aqueous extract was filtered, allowed to cool at room temperature and fed to the rats.

2.4. Quantification of EGCG in WT extract

High-performance liquid chromatography (HPLC) technique was used to determine the concentration of EGCG in WT extract. Briefly, solvent A-10mM sodium phosphate (pH 2.6), and solvent B- acetonitrile (93:7, v/v/) were the mobile phases for the gradient elution. Total EGCG present in the WT extract (Supplementary Figure 1) was then given alone in drinking water to animals.

2.5. In vitro studies

2.5.1. Antioxidant activity

DPPH (2, 2-diphenyl-2-picryl hydrazyl) and ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) assays were employed to determine the antioxidant capacity of WT and EGCG according to the method described by Mahakunakorn et al. (2004). DPPH in its radical form exhibits characteristic absorbance at 515 nm which disappears after its reduction by anti-radical compounds. The antioxidant activity of the sample was expressed as IC50 value.

2.6. In vivo studies

2.6.1. Animals

Female Sprague Dawley (SD) rats weighing 160–250 g were procured from the central animal house facility of Panjab University, Chandigarh, India. The experimental procedures were in compliance with the guidelines of the committee instituted for the purpose of control and supervision of experiments on animal (CPCSEA, India) and approved by the institutional animal ethical committee. Before subjecting the animals to various treatments, the animals were allowed to acclimatize for at least one week. The rats were given standard laboratory pelleted diet and water *ad libitum* throughout the period of experimentation.

2.6.2. Experimental design

Rats were pretreated with WT (1%) or with EGCG (Concentration equivalent to 1% of WT i. e 80 μg/ml) in normal drinking water on alternate days for a period of four weeks. The animals were pretreated with WT and EGCG, and received BaP (50 mg/kg b. wt.) dissolved in olive oil (twice a week) for a period of 4 weeks (5th to 8th week of treatment) (Sikdar et al., 2013). 36 female SD rats were randomly segregated into six experimental groups with six animals per group (Table 1) as per the following schedule:

Group 1, Normal control (NC): The animals in this group served as normal controls and had free access to normal diet and drinking water. The animals received normal drinking water for upto 12th week along with 0.3 ml olive oil orally twice a week from 5th to 8th week.
Group 2, BaP Exposed (BaP): The animals in this group were administered orally with BaP at a dose level of 50mg/kg b. wt. dissolved in olive oil, twice a week, for a duration of 4 weeks (5th to 8th week of treatment) (Sikdar et al., 2013).
Group 3, EGCG controls (EGCG): The animals in this group were treated with EGCG (80 μg/ml) orally on alternate days for entire 12 weeks duration. The dose of EGCG as 80 μg/ml was equivalent to the amount of EGCG present in 1% of WT extract.
Group 4, White tea control (WT): The animals in this group were treated with WT extract (1%) orally on alternate days for the whole 12 weeks.
Group 5, WT + BaP treated (WB): The animals in this group were given WT and BaP at the same doses, as were given to animals belonging to Group 3 and Group 2, respectively. In this group, the treatment with WT started 4 weeks prior to BaP treatment and continued 4 weeks post BaP treatment.
Group 6, EGCG + BaP treated rats (EB): Group 6 animals received a combined treatment of EGCG and BaP at the same doses, as were given to the animals belonging to Group 4 and Group 2, respectively. In this group, the treatment with EGCG started 4 weeks prior to BaP treatment and continued 4 weeks post BaP treatment.

All the animals were sacrificed under light ether anesthesia after 12 weeks by cervical dislocation.

2.6.3. Physical parameters

2.6.3.1. Body weights. The body weights of rats were recorded every week and at the time of sacrifice.

2.6.3.2. Consumption of water, white tea extract and EGCG. The consumption of water, white tea extract and EGCG was recorded every week and expressed as mean consumption/rat.

2.6.4. Biochemical assay

Lungs from all the sacrificed animals were removed and perfused

immediately with 0.9% normal saline (ice cold). 10% homogenates (weight/volume) were prepared from tissue samples in phosphate buffer saline (100 mM; pH 7.4). The biochemical estimations (except GSH & LPO) were carried out in the supernatants obtained after centrifugation of homogenates at 10000 × *g* for 30 min. Protein contents in various samples of crude homogenates and post mitochondrial fragments (PMF) were determined according to the method of Lowry et al. (1951).

2.6.4.1. Oxidative stress biomarker response. The levels of oxidative stress biomarkers which included LPO, PCC, and ROS were determined in lung tissues of normal and different treatment groups. The assay for lipid peroxidation was performed according to the method of Trush and Kensler (1991). The protein carbonyl contents were estimated according to the method of Levine et al. (1994). ROS contents were determined by using probe DCFH-DA which when oxidized gets converted into fluorescent DCF. Briefly, the tissue samples were incubated with 4 µl of DCFH-DA at 37 °C for 30 min in dark. The concentrations of ROS were expressed as relative fluorescence of DCFH-DA.

2.6.4.2. Endogenous antioxidants. The activity of SOD was estimated by following the method as described by Kono (1978). The method described by Luck (1954) was used for the estimation of CAT. Total glutathione levels were estimated in post mitochondrial fractions by using the method of Zahler and Cleland (1968). The reduced glutathione (GSH) content was determined by following the method of Moron et al. (1979). To measure the Redox ratio (GSH/GSSG), oxidized glutathione (GSSG) was calculated from subtracting the total glutathione from reduced glutathione (GSSG = tGSH- GSH). Glutathione-*S*-transferase (GST) activity was calculated by the procedure described by Habig et al. (1974). Glutathione reductase (GR) was estimated in post mitochondrial fractions in accordance with the method of Williams and Arscott (1971). Glutathione peroxidase (GPx) estimation was performed in post mitochondrial fractions as described by previously described method (Flohe, 1985).

2.6.4.3. Inflammatory biomarkers. Nitric oxide levels were measured by using the method of Raddassi et al. (1994). Briefly, 0.2 ml of sample and 0.05 ml of Griess reagent were mixed and incubated in dark for 10 min and then absorbance was noted at 540 nm. The estimation of Citrulline levels in the sample was performed by using the method of Boyde and Rahmatullah (1980).

2.6.5. Histoarchitectural study

Lung tissue samples obtained from treated and respective control animals were subjected to histoarchitectural examination. Small pieces of lung tissue were fixed in 10% formalin and after 8–12 h of fixation; the tissues were thoroughly washed with running water and dehydrated in upgrading concentrations of ethanol (30%, 50%, 70%, 90% and 100%) for 1 h each. After completion of dehydration process, tissues were placed in the mixture of absolute alcohol + benzene (1:1) for 1 h and then in pure benzene for 20 min. After placing in benzene, tissues were subjected to benzene + paraffin wax (1:1) for 1 h and then in pure wax. The tissues were given two changes of wax in 6 h and finally embedded in paraffin wax (60–62 °C). Subsequently, 5 µm thick sections were cut using microtome and slides were made. Histological analyses of liver tissue sections were done by using Hematoxylin and Eosin staining as described by Humanson (1961). The slides were dewaxed in xylene for 10–20 min and were placed in downgrading concentrations of ethanol (absolute 90%, 70%, 50%, and 30% for 2–3 min in each concentration). After hydrating, the slides were rinsed with distilled water and stained in Hematoxylin for one minute then washed in water till the appearance of blue colour. The tissues samples were rinsed in ammonia water and again washed with water. The slides were dipped in acid water if over stained, then dipped in 30% alcohol followed by the 50% and 70% alcohol and stained with 1% alcoholic eosin for one min. The slides were finally rinsed in 90% and 100% alcohol, cleared in xylene and mounted with DPX.

Ten fields in five slides from each group were scored using semi quantitative scoring system by a pathologist blinded to various treatment groups. Variables scored were alveoli inflammation (AI), neutrophil infiltration (NI), alveolar septal thickening (AST), peribronchiolar inflammation (PI), enlargement of alveolar space (ELS) and destruction/fragmentation of septal wall (DSW). Each variable was scored using a 0 to 4 scale, with no injury score as 0, injury in 25% of the field scored 1, injury in 50% of the field scored 2, injury in 75% of the field scored 3 and injury throughout the field scored 4. Maximum possible score was 24. Descriptive statistics (ANOVA followed by LSD post hoc test with significance set at $p < 0.05$) were calculated on all variables and for the percent change observed after lung injury. Data are given as Mean ± SD.

2.6.6. Statistical analysis

The data is expressed in terms of Mean ± SD of six animals for each group. The statistical significance between different groups was performed by using one way analysis of variance (ANOVA). To compare the means among the different treatment groups, post hoc comparisons were performed by least significant difference (LSD) method. The statistical software package SPSS v14 was used and a value of $p \leq 0.05$ was considered to be statistically significant in this study.

3. Results

3.1. In vitro studies

In vitro experiments were conducted to compare the total antioxidant capacity of WT and pure EGCG equivalent to the WT extract by employing DPPH and ABTS free radical scavenging assays and the data is presented in Fig. 1. The results of DPPH assay (Fig. 1) showed concentration dependent scavenging activities of both EGCG and WT with IC50 values 86.14 ± 3.1 µg/ml and 134.97 ± 18.4 µg/ml, respectively. Free radical scavenging activity by ABTS assay was found to be high for EGCG as compared to WT with IC50 values 7.44 ± 1.1 µg/ml and 13.54 ± 3.2 µg/ml, respectively.

3.2. In vivo studies

After analyzing the comparative antioxidant potential of WT and EGCG in scavenging the free radicals in *in vitro* system, we then conducted other experiments to compare the protective potential of EGCG with WT extract against BaP induced pulmonary toxicity. The results from BaP treatment group have been compared with normal control group. Further, the results from Group 3 and 4 were compared with BaP treated animals. Additionally, the results from Group 5 (WB) were also compared with the Group 6 (EB) animals.

3.2.1. Body weight change

The variations in the body weights of the animals amongst various groups were recorded weekly. We observed a steady increase in body weights of the animals among different treatment groups. There were no significant differences in the body weight of the animals subjected to various treatments vis-à-vis normal control animals, throughout the experimental period (data not shown).

3.2.2. Consumption of water, white tea extract and EGCG

The consumption of water, white tea extract and EGCG was also recorded. A significant difference in the consumption of white tea extract and EGCG when compared with water was observed throughout the experimental period. However, this consumption was not significant when EGCG and white tea extract was compared with each other (Fig. 2).

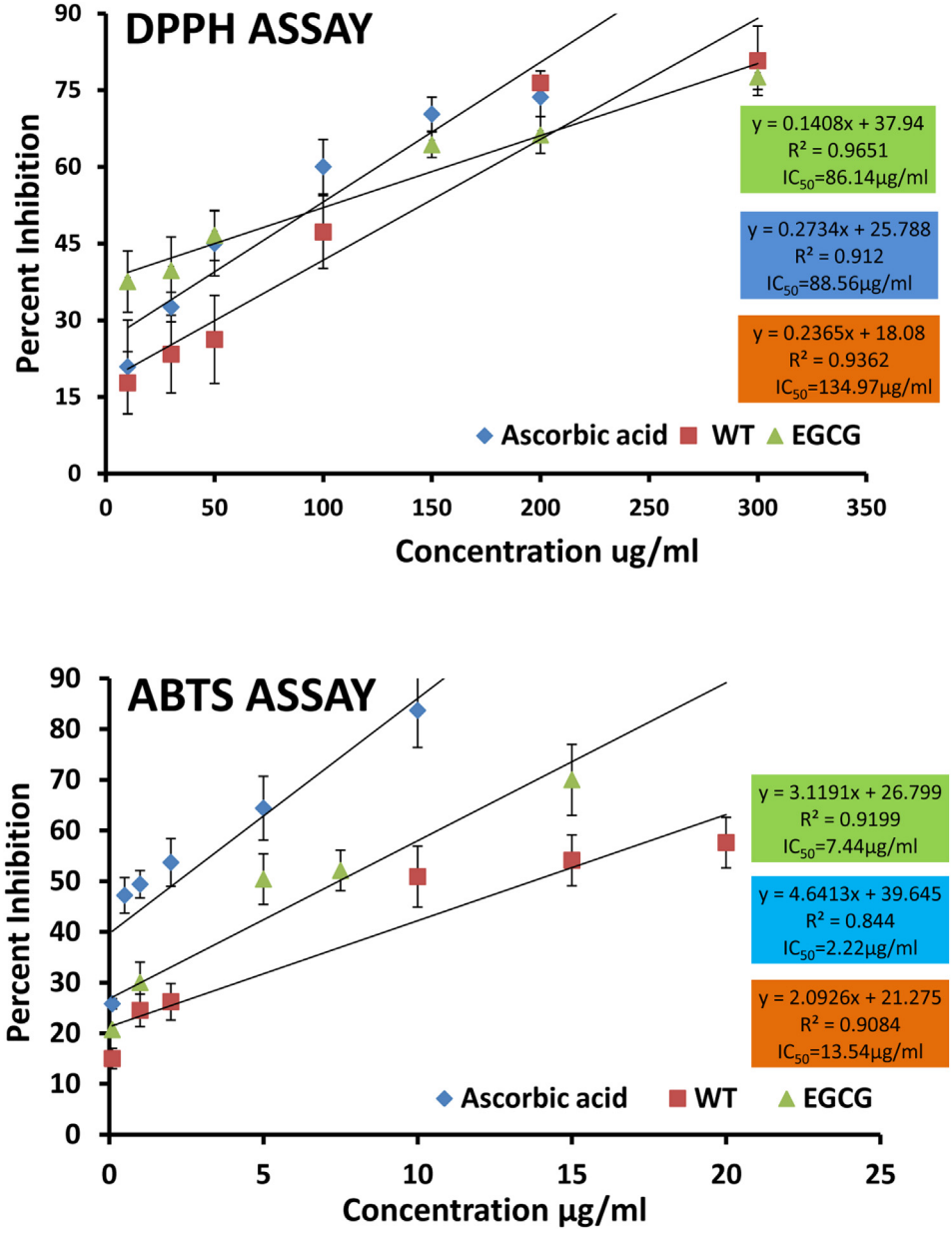

Fig. 1. The total antioxidant capacity of pure EGCG and white tea extract (WT) as measured by DPPH and ABTS assays. The percentage scavenging is plotted against the concentration of the sample. The data are expressed as mean ± SD of three independent assays.

3.2.3. Oxidative stress biomarkers

BaP treatment to normal rats resulted in a significant increase ($p \leq 0.001$) in ROS, LPO and PCC levels in pulmonary tissues in comparison to a normal control group (Fig. 3). However, BaP treated animals which received pure EGCG in drinking water for 4 weeks before and after BaP exposure showed a significant reduction ($p \leq 0.001$) in otherwise elevated levels of ROS, LPO and PCC in pulmonary tissues. Similarly, BaP treated animals supplemented with WT extract also showed a significant reduction ($p \leq 0.001$) in the elevated levels of ROS, LPO and PCC. However, when the levels of oxidative stress biomarkers in group 5 animals (WB) were compared with the group 6 animals (EB), no significant difference in their levels was observed.

3.2.4. Endogenous antioxidants

A statistically significant decrease ($p \leq 0.001$) in the levels of GSH and activities of endogenous antioxidants including SOD, CAT, GR, GPx and GST was observed when normal animals were subjected to BaP treatment (Table 2). Interestingly, when BaP treated animals were administered with EGCG or white tea, the levels of GSH and activities of all these enzymes were elevated significantly ($p \leq 0.001$). Further, the ratio of GSH/GSSG showed a similar trend. When EGCG or white tea was administered to normal rats separately, no significant change in activities of endogenous antioxidants, GSH as well as GSH/GSSG ratio was noticed. Moreover, no significant difference in the levels of endogenous antioxidants was observed when group 5 (WB) animals were compared with the group 6 (EB) thereby implying that White Tea exhibits the same antioxidant efficacy as shown by EGCG.

3.2.5. Inflammatory biomarkers

The levels of inflammatory markers NO and Citrulline in lung tissues were significantly elevated following treatment with BaP, which however were appreciably reduced ($p \leq 0.001$) when supplemented with either EGCG or WT extract (Table 2). When EGCG or white tea were administered to normal rats separately, no significant change in the levels of NO and Citrulline, was noticed.

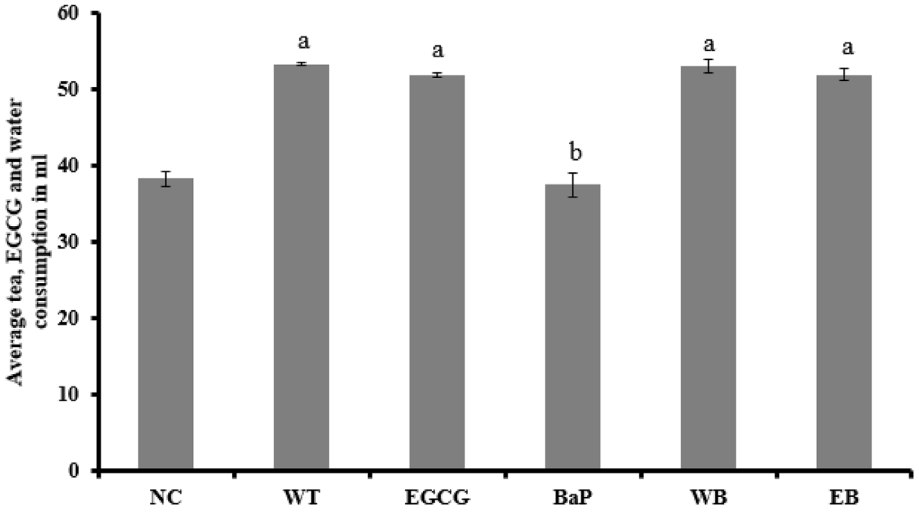

Fig. 2. Water, white tea extract and EGCG consumption among different treatment groups. The values are expressed as Mean ± SD. NC: Normal Control, BaP: Benzo (a)pyrene, EGCG: EGCG treated, WT: White Tea treated, WB: White Tea + Benzo(a) pyrene, and EB: EGCG + Benzo(a)pyrene treated groups. Statistical significance ($p \leq 0.05$), a; as compared with water, b; as compared with white tea extract by ANOVA followed by LSD post hoc test.

3.2.6. Lung histoarchtecture

The histological observations from light microscope preparations of the lungs from EGCG, WT treated animals showed near normal lung histoarchitecture as revealed by normal control animals (Fig. 4). In these animals, the pulmonary alveoli were demarcated by septa composed of a continuous layer of epithelial cells overlying a thin interstitium. The two morphologically distinct cells -type I and type II alveolar cells with large nuclei, reduced cytoplasm and microvilli were also present. Normal alveolar spaces and wide air sacculi were also observed. BaP intoxicated lungs displayed thickening of alveolar septa and hypertrophied alveolar cells. The important morphological changes including alveolar septal thickening, inflammatory cell infiltration and epithelial cell detachment of bronchi and bronchioles were observed. Moreover, some areas of lungs showed dilation of many alveolar spaces and destruction of the septal walls. The affected alveoli were irregular which lost the normal alveolar structure. The bronchiolar epithelium showed appreciable changes involving thinned alveolar wall and degenerated cartilage in pulmonary tissues. The animals intoxicated with BaP also had highest total lung tissue injury index ($P < 0.05$) (Table 3). The lungs of BaP exposed animals when co-administered with pure EGCG and WT showed similar morphological features as they appeared in the normal control lungs except that mild interalveolar

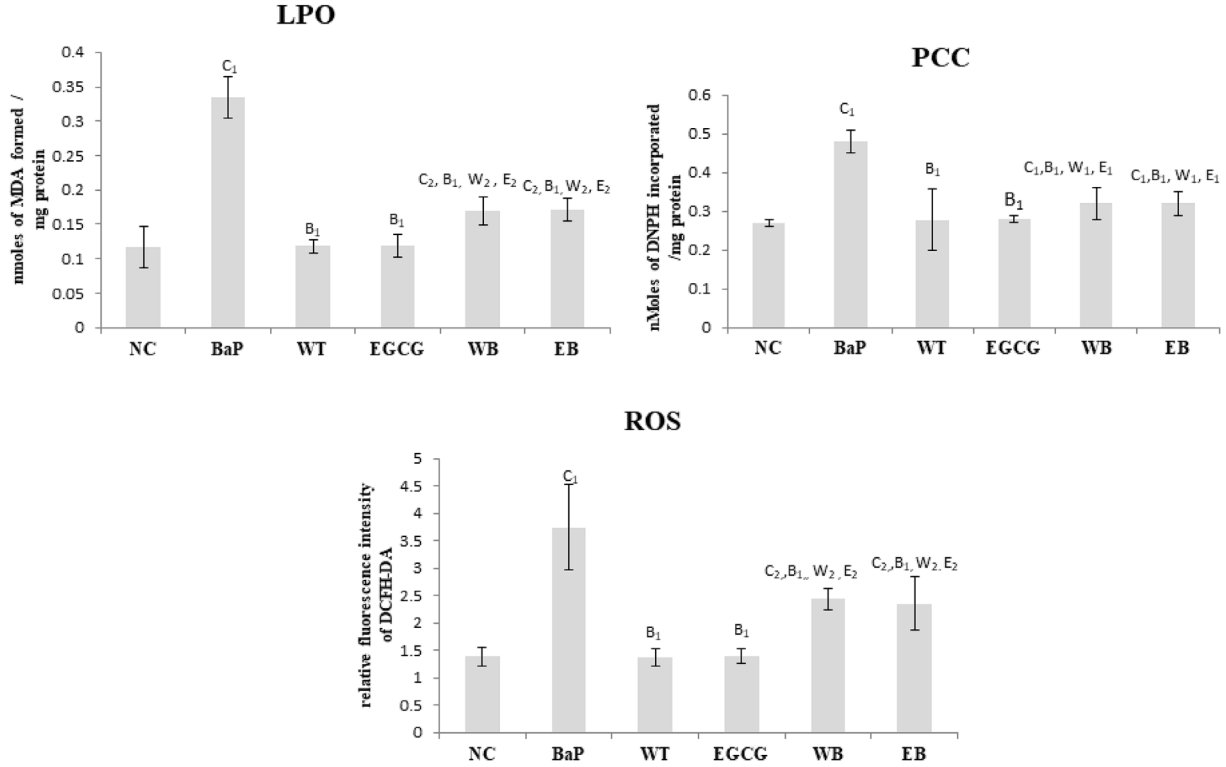

Fig. 3. Effects of pure EGCG and white tea extract (WT) on the extent of lipid peroxidation (LPO), protein carbonyl content (PCC) and total reactive oxygen species (ROS) in pulmonary tissue of benzo(a)pyrene treated rats. The values are expressed as Mean ± SD; N = 6. NC: Normal Control, BaP: Benzo (a) pyrene, EGCG: EGCG treated, WT: White Tea treated, WB: White Tea + Benzo(a)pyrene, and EB: EGCG + Benzo(a)pyrene treated groups. $C_1 = p \leq 0.001$, $C_2 = p \leq 0.01$, $C_3 = p \leq 0.05$, by post hoc analysis when values are compared with normal control animals. $B_1 = p \leq 0.001$, $B_2 = p \leq 0.01$, $B_3 = p \leq 0.05$, by post hoc analysis when values are compared with benzo(a)pyrene group. $E_1 = p \leq 0.001$, $E_2 = p \leq 0.01$,$E_3 = p \leq 0.05$, by post hoc analysis when values are compared with EGCG group. $W_1 = p \leq 0.001$, $W_2 = p \leq 0.01$, $W_3 = p \leq 0.05$, by post hoc analysis when values are compared with White tea group.

Table 2
Effect of pure EGCG and White Tea extract (WT) on endogenous antioxidants in pulmonary tissues, nitric oxide and citrulline content in the liver of BaP treated rats. All the values are expressed as Mean ± S.D., n = 6. NC: Normal Control, BaP: Benzo (a) pyrene treated, EGCG: EGCG treated, WT: White Tea treated, WB: White Tea + Benzo (a) pyrene treated, and EB: EGCG + Benzo(a)pyrene treated groups.

Groups/Parameters	NC	BaP	EGCG	WT	WB	EB
NO (μ Moles of Nitrite accumulated/g Tissue)	0.3 ± 0.23	0.55 ± 0.52 C1	0.30 ± 0.21 B1	0.31 ± 0.18 B1	0.37 ± 0.21 C2, B1, E1, W2	0.36 ± 0.16 C2, B1, E2, W2
Citrulline (n Moles of L-Citrulline/g Tissue)	21.31 ± .97	34.01 ± 3.03 C1	20.04 ± 2.44 B1	20.21 ± 1.30 B1	24.80 ± 3.69 B1, E3, W3	23.99 ± 1.98 B1, E3, W3
GSH (μ Moles of reduced Glutathione/g Tissue)	1.68 ± 0.15	0.89 ± 0.10 C1	1.68 ± 0.08 B1	1.66 ± 0.07 B1	1.39 ± 0.05 C1, B1,E1, W1	1.40 ± 0.04 C1, B1, E1, W1
GSSG/GSH	1.68 ± 0.035	3.71 ± 0.047 C1	1.71 ± 0.03 B1	1.70 ± 0.06 B1	2.01 ± 0.090 C1, B1,E1,W1	1.96 ± 0.10 C1, B1,E1,W1
GR (n Moles of NADPH oxidized/min/mg Protein)	42.70 ± 5.7	28.32 ± 5.6 C2	18.42 ± 5.5 C1, B3	17.88 ± 2.2 C1, B3	22.87 ± 7.2 C1	14.47 ± 1.45 C1,B2
GPx (n Moles of NADPH oxidized/min/mg Protein)	30.38 ± 5.75	22.06 ± 4.03	29.21 ± 5.7	32 ± 10 B3	25.58 ± 2.47	1.97 ± 2.45 C3, W3
GST (μ Moles of GSH-CDNB conjugate formed/min/mg Protein)	0.107 ± 0.04	0.047 ± 0.003	0.127 ± 0.01 B1	0.116 ± 0.01 B1	0.065 ± 0.013 C3, E1,W2	0.067 ± 0.01 C3,E1,W2
SOD (IU/mg Protein)	12.48 ± 0.55	8.12 ± 0.39 C1	12.39 ± 0.76 B1	12.27 ± 0.78 B1	10.80 ± 0.90 C2, B1, E3,W3	10.87 ± 0.64 C2, B1, E3,W3
Catalase (m Moles of MDA formed/min/mg Protein)	0.93 ± 0.10	0.44 ± 0.76 C1	0.92 ± 0.12 B1	0.91 ± 0.06 B1	0.710 ± 0.06 C2, B1, E2, W2	0.712 ± 0.06 C2, B1, E2, W2

$C_1 = p \leq 0.001$, $C_2 = p \leq 0.01$, $C_3 = p \leq 0.05$, by post hoc analysis when values are compared with normal control animals.
$B_1 = p \leq 0.001$, $B_2 = p \leq 0.01$, $B_3 = p \leq 0.05$, by post hoc analysis when values are compared with benzo(a)pyrene group.
$E_1 = p \leq 0.001$, $E_2 = p \leq 0.01$,$E_3 = p \leq 0.05$, by post hoc analysis when values are compared with EGCG group.
$W_1 = p \leq 0.001$, $W_2 = p \leq 0.01$, $W_3 = p \leq 0.05$, by post hoc analysis when values are compared with White tea group.

septal thickening and peribronchioler inflammation were present. Similar observations were also recorded in tissue injury index (Table 3).

4. Discussion

The primary objective of the present study was to understand whether pure EGCG affords the same protection as provided by WT extract against BaP induced toxicity in pulmonary tissues of the rats.

The validated markers of oxidative stress such as GSH, LPO, PCC and ROS (Toyokuni et al., 1995) which were elevated in BaP animals were decreased upon supplementation with WT and pure EGCG. These results do suggest the protective efficacy of both WT and EGCG in mitigating the BaP induced oxidative stress. The previous studies on white tea had also documented similar findings (Kumar et al., 2012a,b). The alteration in redox homeostasis is a crucial step in BaP induced toxicity (Alvarez-Gonzalez et al.; Kasala et al., 2015). So, we also

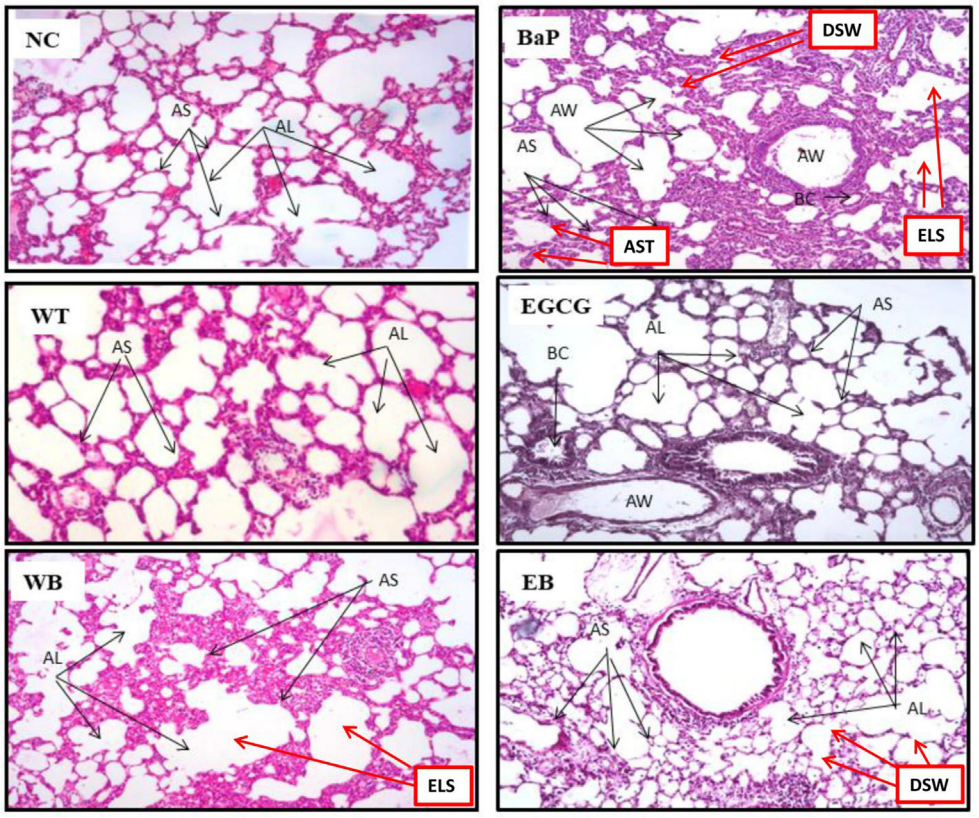

Fig. 4. Histological assessment of pulmonary morphology at 12 weeks. Light microscopic images of representative paraffin-embedded lung sections from different treatment groups stained with hematoxylin and eosin (H&E). NC: Normal Control, BaP: Benzo (a) pyrene, EGCG: EGCG treated, WT: White Tea treated, WB: White Tea + Benzo (a) pyrene, and EB: EGCG + Benzo(a) pyrene treated groups. AS: Alveolar spaces, AW: Airway, AL: Alveoli, BC: Bronchioles, PI: Peribroncheolar Inflammation, ELS: Enlargement of alveolar space, DSW: Destruction/fragmentation of septal walls.

Table 3
Individual lung injury scores. NC: Normal Control, BaP: Benzo (a) pyrene treated, EGCG: EGCG treated, WT: White Tea treated, WB: White Tea + Benzo (a)pyrene treated, and EB: EGCG + Benzo(a)pyrene treated groups. Descriptive statistics (ANOVA followed by LSD post hoc test) calculated and the values are expressed as Mean ± S.D., n = 5. Statistical significance set $p < 0.05$ where; a as compared with NC; b as commpared with BaP; c as compared with WB group.

Individual lung score	NC	BaP	EGCG	WT	WB	EB
Alveolar inflammation (AI)	0.6 ± .5	3.8 ± .4[a]	0.2 ± .4[b]	0.4 ± .5[b]	1.8 ± .8[a,b]	1.6 ± .5[a,b]
Neutrophil infiltration (NI)	0.0	1.6 ± .8[a]	0.2 ± .4[b]	0.2 ± .4[b]	0.2 ± .4[b]	0.0[b]
Alveolar septal thickening (AST)	0.4 ± .5	2.2 ± 1.6[a]	0.6 ± .8[b]	0.6 ± .8[b]	1.2 ± .8[b]	1.2 ± .4[b]
Peribronchiolar inflammation (PI)	0.6 ± .5	3.4 ± .8[a]	0.4 ± .5[b]	0.6 ± .5[b]	0.6 ± .5[b]	0.4 ± .5[b]
Enlargement of laveolar spaces (ELS)	0.2 ± .4	1.8 ± .4[a]	0.2 ± .4[b]	0.0[b]	0.4 ± .5[b]	0.6 ± .5[b]
Destruction/fragmentation of septal walls (DSW)	0.0	2.8 ± .4[a]	0.0[b]	0.0[b]	0.4 ± .8[b]	0.2 ± .4[b]
Total Score	**1.8**	**15.6**	**1.6**	**1.8**	**4.6**	**4**

determined the total ROS levels as well as the activities of major endogenous antioxidants (SOD, CAT, GR, GST, and GPx) in different treatment groups. The higher levels of ROS and decreased endogenous antioxidants (SOD, CAT, GR, GST, GPx and GSH) clearly indicated the imposition of oxidative stress in BaP exposed animals. However, reduction in oxidative stress was observed as evidenced by increased levels of endogenous antioxidants (SOD, CAT, GR, GST, GPx and GSH) following separate supplementation of EGCG and WT to BaP treated animals.

The oxidative degradation of fatty acids affects the integrity of plasma membrane, which in turn can alter its fluidity and also affects the normal functioning of associated enzymes (Brrera, 2012). Beside, oxidation of proteins resulting in elevated content of PCC (Stadtman, 1990), sulfur atoms in amino acid are also susceptible to oxidation (Shacter, 2000), which in turn lead to alterations in the conformation of secondary and tertiary structures of proteins (Zhang et al., 2013). Oxidation further alters the proteolytic properties of proteins (Morzel et al., 2006). Our results clearly demonstrate that pure EGCG and WT effectively modulate the activities of endogenous antioxidative enzymes and exhibit carbonyl scavenging properties as evidenced by low level of ROS, LPO and PCC in BaP exposed animals. But, when EGCG + BaP group was compared with the group that received WT treatment along with BaP exposure, no significant change in the outcome was observed; thereby suggesting that both WT and EGCG have more or less the same protective potential to inhibit BaP induced pulmonary toxicity.

GSH is an important reductant in our body which protects the cellular components from ROS induced damage. The oxidized form of glutathione (GSSG) is converted back to reduced form (GSH) by GR with the help of NADPH (Mateen et al., 2016). The low levels of GSH/GSSG and reduced activities of endogenous antioxidant enzymes viz., SOD, CAT, GST and GR as observed in pulmonary tissues of BaP exposed animals have also been reported in previous studies (Bodduluru et al., 2016; Kumar et al., 2017; Sehgal et al., 2013). Additionally, we observed an appreciable improvement in the activities of above said antioxidants in animals co supplemented with pure EGCG and WT extract. Interestingly, we did not observe any difference in the above biochemical indices when EGCG treated group was compared with WT supplemented group. Consequently, the results from *in vivo* study do suggest that both EGCG in pure form and WT extract exert indistinguishable protection against BaP induced pulmonary toxicity.

The levels of NO and citrulline (inflammatory markers) have been reported to be increased during chronic inflammations (Geller et al., 1993). We also noticed an increase in these indices in BaP treated animals which is arguably a response of pulmonary tissues against inflammatory inputs. The levels of nitric oxide also get increased in other medical conditions including cirrhosis (Pilette et al., 1996). Literature is replete with reports that advocate the potential of tea catechins to decrease the nitric oxide production (Nomura et al., 2017; Wang et al., 2017; Williamson, 2017). Earlier, it was proposed that phytochemicals with NO scavenging potential may have an important role to exert protection against inflammatory responses (Majano et al., 2004). Our results also make obvious that pure EGCG and WT supplementation to BaP exposed animals reduced the NO and citrulline levels, hence suggesting the protective role of both EGCG and WT in pulmonary toxicity.

The histoarchitectural analysis of the current study clearly showed the uniform arrangement of lung alveolar spaces with regular arranged small nuclei-filled cells in normal and EGCG as well as WT treated lung sections. However, the toxic effects of BaP on rat lung alveolar cells were evidenced by deformation in alveolar cells and reduced interstitial spaces due to thickening of alveolar walls. Lipid peroxidation in the cell membranes of alveolar wall due to oxidative stress is associated with cellular damage which may possibly be prevented by antioxidants. In our study, both WT extract and EGCG, show considerable histoarchitectural improvements in pulmonary tissues of BaP intoxicated animals. Further, increased levels of LPO, PCC support histological impairment of lung tissue in BaP treated animals. Besides, simultaneous co-administration of EGCG and WT to BaP exposed animals showed melioration and revealed near normal histoarchitecture of BaP induced altered pulmonary tissue.

However, the most important outcome of the present study is that we did not find any noticeable difference in the improvement potential of WT extract vis a vis EGCG equivalent to the EGCG present in the WT extract in modulating the BaP induced altered biochemical indices and pulmonary histoarchitecture which is contrary to *in-vitro* studies. This may be possible, as the white tea extract contain other polyphenols which may aid in the increased absorption of EGCG thus improving the bioavailability of EGCG.

5. Conclusion

Since, isolation of EGCG is cumbersome and costly procedure and especially when both white tea and EGCG exhibit similar antioxidative efficacy in containing damage inflicted by BaP on pulmonary tissue, then white tea can serve as a cost-effective alternative to EGCG. Though, the present study espouses the prospective roles of EGCG and White tea extract as protective measures to limit the oxidative damage following BaP induced pulmonary toxicity (Higdon et al., 2003), but the possible contribution of other catechins towards antioxidant potential of white tea in lungs cannot be ruled out.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors are grateful to Department of Biophysics and UGC India, for providing financial assistance in the form of Post-Doctoral Fellowship (DSKPDF) (4-2/2006(BSR)/BL/13-14/0353 to Dr. Sunil Kumar Dhatwalia. Authors are also grateful to Dr. Saakshi Verma for her kind help to determine lung tissue injury index.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.05.059.

References

Bodduluru, L.N., Kasala, E.R., Madhana, R.M., Barua, C.C., Hussain, M.I., Haloi, P., Borah, P., 2016. Naringenin ameliorates inflammation and cell proliferation in benzo (a) pyrene induced pulmonary carcinogenesis by modulating CYP1A1, NFkappaB and PCNA expression. Int. Immunopharmacol. 30, 102–110.

Boyde, T.R.C., Rahmatullah, M., 1980. Optimization of conditions for the colorimetric determination of citrulline, using diacetyl monoxime. Anal. Biochem. 107, 424–431.

Brrera, G., 2012. Oxidative stress and lipid peroxidation products in cancer progression and therapy. ISRN Oncol 1–23 2012.

Cao, Z., Wang, Y., Ma, Y., Xu, Z., Shi, G., Zhuang, Y., Zhu, T., 2005. Occurrence and distribution of polycyclic aromatic hydrocarbons in reclaimed water and surface water of Tianjin, China. J. Hazard Mater. 122, 51–59.

Chen, D., Wan, S.B., Yang, H., Yuan, J., Chan, T.H., Dou, Q.P., 2011. EGCG green tea polyphenols and their synthetic analogs and prodrugs for human cancer prevention and treatment. Adv. Clin. Chem. 53, 155–177.

Chhabra, S.K., Yang, C.S., 2001. Tea and prostate cancer. Epidemiol. Rev. 23, 106–109.

Chiarugi, P., Fiaschi, T., 2007. Redox signalling in anchorage-dependent cell growth. Cell. Signal. 19, 672e682.

Flohe, L., 1985. The glutathione peroxidase reaction: molecular basis of the antioxidant function of selenium in mammals. Curr. Top. Cell. Regul. 27, 473e478.

Fujiki, H., Suganuma, M., Imai, K., Nakachi, K., 2002. Green tea: cancer preventive beverage and/or drug. Cancer Lett. 188, 9–13.

Geller, D.A., Nussler, A.K., Di Silvio, M., Lowenstein, C.J., Shapiro, R.A., Wang, S.C., Simmons, R.L., Billiar, T.R., 1993. Cytokines, endotoxin, and glucocorticoids regulate the expression of inducible nitric oxide synthase in hepatocytes. Proc. Natl. Acad. Sci. U.S.A. 90, 522e526.

Habig, W.H., Pabst, M.J., Fleischner, G., Gatmaitan, Z., Arias, I.M., Jakoby, W.B., 1974. The identity of glutathione S-transferase B with ligandin, a major binding protein of liver. Proc. Natl. Acad. Sci. U.S.A. 71, 3879e3882.

Humanson, G.L., 1961. Basic procedures: Animal tissue technique. Anim. Tissue Tech. 130–132.

Higdon, J.V., Frei, B., 2003. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. Crit. Rev. Food Sci. Nutr. 43, 89–143.

Kasala, E.R., Bodduluru, L.N., Barua, C.C., Sriram, C.S., Gogoi, R., 2015. Benzo (a) pyrene induced lung cancer: role of dietary phytochemicals in chemoprevention. Pharmacol. Rep. 67, 996e1009.

Kono, Y., 1978. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch. Biochem. Biophys. 186, 189e195.

Kumar, M., Jain, M., Sehgal, A., Sharma, V.L., 2012a. Modulation of CYP1A1, CYP1B1 and DNA adducts level by green and white tea in Balb/c mice. Food Chem. Toxicol. 50, 4375e4381.

Kumar, M., Sharma, V.L., Sehgal, A., Jain, M., 2012b. Protective effects of green and white tea against benzo(a)pyrene induced oxidative stress and DNA damage in murine model. Nutr. Canc. 64, 300e306.

Kumar, M., Singh, G., Bhardwaj, P., Dhatwalia, S.K., Dhawan, D.K., 2017. Understanding the role of 3-O-Acetyl-11-keto-β-boswellic acid in conditions of oxidative-stress mediated hepatic dysfunction during benzo (a) pyrene induced toxicity. Food Chem. Toxicol. https://doi.org/10.1016/j.fct.2017.03.058.

Levine, R.L., Williams, J.A., Stadtman, E.R., Shacter, E., 1994. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol. 233, 346e357.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265e275.

Luck, H., 1954. Quantitative determination of catalase activity of biological material. Enzymologia 17, 31e40.

Mahakunakorn, P., Tohda, M., Murakami, Y., Matsumoto, K., Watanabe, H., 2004. Antioxidant and free radical-scavenging activity of Choto-san and its related constituents. Biol. Pharm. Bull. 27, 38e46.

Majano, P.L., Medina, J., Zubia, I., Sunyer, L., Lara-Pezzi, E., Maldonado-Rodriguez, A., Lopez-Cabrera, M., Moreno-Otero, R., 2004. N-Acetyl-cysteine modulates inducible nitric oxide synthase gene expression in human hepatocytes. J. Hepatol. 40, 632e637.

Mateen, S., Moin, A., Khan, A., Zafer, A., Fatima, N., 2016. Increased reactive oxygen species formation and oxidative stress in rheumatoid arthritis. PLoS One. https://doi.org/10.1371/journal.pone.0152925.

Moron, M.S., Depierre, J.W., Mannervik, B., 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim. Biophys. Acta 582, 67e78.

Morzel, M., Gatellier, P., Sayd, T., Renerre, M., Laville, E., 2006. Chemical oxidation decreases proteolytic susceptibility of skeletal muscle myofibrillar proteins. Meat Sci. 73, 536e543.

Nomura, S., Monobe, M., Ema, K., Maeda-Yamamoto, M., Nesumi, A., 2017. Comparison of the effects of three tea cultivars (Camellia sinensis L.) on nitric oxide production and aortic soluble guanylate cyclase expression in high-salt diet-fed spontaneously hypertensive rats. J. Nutr. Sci. Vitaminol. 63, 306–314. https://doi.org/10.3177/jnsv.63.306.

Penning, T.M., Burczynski, M.E., Hung, C.F., McCoull, K.D., Palackal, N.T., Tsuruda, L.S., 1999. Dihydrodiol dehydrogenases and polycyclic aromatic hydrocarbon activation: generation of reactive and redox active o-quinones. Chem. Res. Toxicol. 12, 1e18.

Peschard, P., Park, M., 2007. From Tpr-Met to Met, tumorigenesis and tubes. Oncogene 26, 1276–1285.

Pilette, C., Kirstetter, P., Sogni, P., Cailmail, S., Moreau, R., Lebrec, D., 1996. Dosedependent effects of a nitric oxide biosynthesis inhibitor on hyperdynamic circulation in two models of portal hypertension in conscious rats. J. Gastroenterol. Hepatol. 11, 1e6.

Poirier, M.C., 2004. Chemical-induced DNA damage and human cancer risk. Nat.Rev. Cancer 4, 630e637.

Raddassi, K., Berthon, B., Petit, J.F., Lemaire, G., 1994. Role of calcium in the activation of mouse peritoneal macrophages: induction of NO synthase by calcium ionophores and thapsigargin. Cell. Immunol. 153, 443–455.

Rhee, S.G., Bae, Y.S., Lee, S.R., Kwon, J., 2000. Hydrogen peroxide: a key messenger that modulates protein phosphorylation through cysteine oxidation. Sci. STKE. https://doi.org/10.1126/stke.2000.53.pe1.

Sehgal, A., Kumar, M., Jain, M., Dhawan, D.K., 2013. Modulatory effects of curcumin in conjunction with piperine on benzo(a)pyrene-mediated DNA adducts and biotransformation enzymes. Nutr. Canc. 65, 885e890.

Shacter, E., 2000. Quantification and significance of protein oxidation in biological samples. Drug Metab. Rev. 32, 307e326.

Siddiqui, I.A., Asim, M., Hafeez, B.B., Adhami, V.M., Tarapore, R.S., Mukhtar, H., 2011. Green tea polyphenol EGCG blunts androgen receptor function in prostate cancer. FASEB J. 25, 1198–1207. https://doi.org/10.1096/fj.10-167924.

Sikdar, S., Mukherjee, A., Bishayee, K., Paul, A., Saha, S.K., Ghosh, S., KhudaBukhsh, A.R., 2013. Post-cancer treatment with condurango 30C shows amelioration of Benzo[a] pyrene-induced lung cancer in rats through the molecular pathway of Caspa- se-3-mediated apoptosis induction: -anti-lung cancer potential of Condurango 30C in rats. J. Pharmacopuncture 16, 11e22.

Srivastava, S.K., Xia, H., Pal, A., Hu, X., Guo, J., Singh, S.V., 2000. Potentiation of benzo [a]pyrene-induced pulmonary and forestomach tumorigenesis in mice by D,Lbuthionine-S,R-sulfoximine-mediated tissue glutathione depletion. Cancer Lett. 153, 35e39.

Stadtman, E.R., 1990. Metal ion-catalyzed oxidation of proteins: biochemical mechanism and biological consequences. Free Radic. Biol. Med. 9, 315e325.

Storz, P., 2005. Reactive oxygen species in tumor progression. Front. Biosci. 10, 1881e1896.

Toyokuni, S., Okamoto, K., Yodoi, J., Hiai, H., 1995. Persistent oxidative stress in cancer. FEBS Lett. 358, 1e3.

Trush, M.A., Kensler, T.W., 1991. An overview of the relationship between oxidative stress and chemical carcinogenesis. Free Radic. Biol. Med. 10, 201e209.

Unachukwu, U.J., Ahmed, S., Kavalier, A., Lyles, J.T., Kennelly, E.J., 2010. White and green tea (Camellia sinensis var. sinensis): variation in phenolic, methylxanthine, and antioxidant profiles. J. Food Sci. 75https://doi.org/10.1111/j.1750-3841.2010.01705.x. C541-8.

Wang, D., Gao, Q., Wang, T., Zhao, G., Qian, F., Huang, J., Wang, H., Zhang, X., Wang, Y., 2017. Green tea infusion protects against alcoholic liver injury by attenuating inflammation and regulating the PI3K/Akt/eNOS pathway in C57BL/6 mice. Food Funct 20, 3165–3177. https://doi.org/10.1039/c7fo00791d.

Williams, C.M., Arscott, I.D., 1971. Glutathione reductase (1971). Methods Enzymol. XVIIB 503–509.

Williamson, G., 2017. The role of polyphenols in modern nutrition. Nutr. Bull. 42, 226–235. https://doi.org/10.1111/nbu.12278.

Zahler, W.L., Cleland, W.W., 1968. A specific and sensitive assay for disulfides (1968). J. Biol. Chem. 243, 716–719.

Zhang, M., Holman, C.D., Huang, J.P., Xie, X., 2007. Green tea and the prevention of breast cancer: a case-control study in Southeast China. Carcinogenesis 28, 1074–1078.

Zhang, W., Xiao, S., Ahn, D.U., 2013. Protein oxidation: basic principles and implications for meat quality. Crit. Rev. Food Sci. Nutr. 53, 1191e1201.