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Suppression of free-radicals and protection against H_2O_2-induced oxidative damage in HPF-1 cell by oxidized phenolic compounds present in black tea

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Abstract

Whereas catechins are the most abundant polyphenols in green tea, the typical pigments in black tea are theaflavins (TF), thearubigins (TR) and theabrownins (TB), which are derived from the oxidation of catechins and their gallates during the fermentation stage of black tea processing. In this study, oxidized phenolic compounds (TF, TR and TB) present in black tea were obtained from a model oxidation system, using immobile enzyme. The order of $OH^{\cdot -}$ and 2,2-diphenyl-1-picrylhydrazyl-scavenging ability of these oxidation products was TF > TB > TR. These oxidized phenolic compounds showed protection against H_2O_2-induced damage in HPF-1 cells and suppressed the accumulation of intracellular reactive oxygen species in H_2O_2-induced damage of HPF-1 cells. Interestingly, TB, as a further oxidative product from TF or TR, showed potent activity, followed TF in the above four systems.

Keywords: Antioxidant activity; Theaflavins; Thearubigins; Theabrownins; Oxidative damage; HPF-1

1. Introduction

During the past decade, numerous studies have suggested possible effects of green and black teas and their polyphenolic constituents as antioxidants and *in vitro* and *in vivo* models of risk of cancer and cardiovascular disease or markers of lipid metabolism (Bushman, 1998; Luczaj & Skrzydlewska, 2005; Trevisanato, 2000; Zhu, Huang, & Tu, 2005). Whereas catechins are the most abundant polyphenols in green tea, the typical pigments in black tea are theaflavins (TF), thearubigins (TR) and theabrownins (TB), which are derived from the oxidation of catechins and their gallates during the fermentation stage of black tea processing (Wan, 2003).

TF is orange or orange-red in colour and possesses a benzotropolone skeleton (Roberts, 1958). It is known that TF, which accounts for 2–6% of the dry weight of solids in brewed black tea (Balentine, Wiseman, & Bouwens, 1997), contributes importantly to properties such as colour, 'mouthfeel' and extent of tea cream formation of black tea (Millin, Crispin, & Swaine, 1969; Powell, Clifford, Opie, Robertson, & Gibson, 1992; Roberts, 1962b). TF has shown strong antioxidant activity against LDL oxidation in mouse macrophage cells (Yoshida et al., 1999), and acts as a preventive of DNA oxidative damage in cell-free

Abbreviations: CBSS, carbonic acid buffered saline solution; CL, chemiluminescence intensity; DCF-DA, 2′,7′-dichlorofluorescein diacetate; DMEM, Dulbecco's modified eagle medium; DMSO, dimethyl sulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EGCG, epigallocatechin gallate; HPF-1, embryonic human lung fibroblasts; HPS, Hallam's physiological saline; IC_{50}, 50%-inhibition concentrations; MTT, 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazoliumbromide; POD, peroxidase; PPO, polyphenol oxidase; ROS, reactive oxygen species; SIPS, stress-induced premature senescence; TB, theabrownins; TF, theaflavins; TR, thearubigins.

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systems (Shiraki et al., 1994), in the inhibition of xanthine oxidase and suppression of intracellular reactive oxygen species in HL-60 cells (Lin, Chen, Ho, & Lin-Shiau, 2000). TR is the most abundant of black tea polyphenols, at approximately 12–18% of solid extracts of black tea liquors (Graham, 1992). It embraces a number of indeterminate structures, from the 'monomeric' to the 'polymeric', derived by enzymatic oxidation of the flavan-3-ols (Haslam, 2003). It is rust-brown in colour and contributes the reddish colour and richness in taste, totally termed 'body' to black tea (Roberts, 1962a). Recently, TR has attracted considerable interest because of its beneficial health properties, including antiproliferative effect on A432 cells and mouse NIH3T3 fibroblast cells (Liang et al., 1999), inhibition of intestinal carcinogenesis (Lodovici et al., 2000), antimutagenic effects *in vitro* (Catterall, Copeland, Clifford, & Ioannides, 1998; Gupta, Chaudhuri, Ganguly, & Giri, 2001; Halder, Pramanick, Mukhopadhyay, & Giri, 2005;), antileukemic effects in U-937 cell and leukemic cells (Das et al., 2002), anti-inflammatory (Maity et al., 2003), antineurotoxin effects in mice (Satoh, Ishii, Shimizu, Sawamura, & Nishimura, 2001, 2002), and inhibition of lipid peroxidation under biological conditions (Yoshino, Hara, Sano, & Tomita, 1994). However, little progress has been made toward an understanding of the chemical nature of the TR. Unlike the well-characterised TF and TR, TB is very poorly characterized. TB is brown and very soluble in water. At best, TB may be taken as a term to embrace amylose, protein, nucleic acid and polyphenols, derived from the oxidation of TF and TR as a result of the excessive withering and anoxic fermentation during black tea processing (Wan, 2003). TB endows tea liquor and leaf with a dark brown colour, which has a negative effect on tea quality (Yuan, 1983).

Polyphenoloxidase (PPO) and peroxidase (POD) are key enzymes for pigment generation during black tea processing (Dix, Fairley, Millin, & Swaine, 1981). The *in vitro* study of tea PPO-catalysed formation of black tea oxidation products has been carried out by a number of researchers (Bonnely, Davis, Lewis, & Astill, 2003; Opie, Clifford, & Robertson, 1993, 1995; Robertson, 1983a, 1983b; Tu, Xu, Xia, & Watanabe, 2005). Model oxidation systems have also been used to compare the oxidation products obtained with tea PPO and with horseradish POD. Whereas more TF was obtained from PPO oxidation of catechins, more TR was obtained from POD oxidation of TF (Finger, 1994). It was elucidated that oxidized phenolic compounds from immobile enzyme systems were similar to those already described in black tea using HPLC-UV and ^{1}H NMR analysis (Bonnely et al., 2003).

In this study, oxidized phenolic compounds (TF, TR and TB) present in black tea were obtained from a model oxidation system, using immobile enzyme. The antioxidant activities of these oxidation products were evaluated in a Fenton reaction system, 2,2-diphenyl-1-picrylhydrazyl (DPPH) system and H_2O_2-induced oxidative damage system in HPF-1 cells (Frippiat et al., 2001). Their antioxidant mechanisms were considered.

2. Materials and methods

2.1. Chemicals

DPPH, Trypsin, penicillin, streptomycin, 2′,7′-dichlorofluorescin diacetate (DCF-DA) and 3-aminophthalhydrazide (Luminol) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco's modified eagle medium (DMEM), newborn calf serum, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliunbromide (MTT) were purchased from GIBCO BRL (Grand Island, NY, USA). Epigallocatechin gallate (EGCG) was a generous gift from the Tea Department of Zhejiang University, the purity was above 98% (analyzed by HPLC). All other chemicals made in China were of analytical grade.

2.2. Preparation of TF, TR and TB

TF was prepared by enzymatic oxidation of tea polyphenols (80% purity, provided by Siming Natural Plant Co., Zhejiang, China) using immobilized PPO. The procedures to produce TF were similar to that described in the patent (Tu & Xia, 2004; Tu, Tang, & Watanabe, 2004). Sodium alginate was used for PPO entrapment; 75 ml of PPO (1500 u) solution were mixed with 100 ml of sodium alginate solution (2%) and entrapped for 5 min; then the enzyme mixture was injected into 1000 ml of 0.1 M $CaCl_2$ solution (by injector). After shaking for 30 min, the particles were taken out and kept in 0.025% glutaraldehyde aqueous for 1 h; an insoluble aggregate between PPO and cross-linking reagent was formed and kept in the pH 5.6 citrate buffer at 4 °C. The oxidation reaction system contained 500 ml of tea polyphenols (5.95 mg/ml) and 15 g of immobilized PPO. Reaction was carried out at defined pH of 5.6 at 37 °C for 30 min with a stirrer; then immobilized PPO was removed by filtering and TF solution was obtained. Ten grams of fresh tea leaves and 2 ml of hydrogen peroxide (1.5%, v/v) (Bonnely et al., 2003) were added to 100 ml of TF solution. The pH value of the mixture was adjusted to 8 and it was stirred using a IKA Labortechnik Stirrer (speed 2) at 37 °C for 45 min. The mixture was heated to destroy enzyme activity (to prevent the further oxidation) and was extracted by ethyl acetate when it reached room temperature. The ethyl acetate fraction was concentrated under vacuum to remove the organic solvent to give a rust-brown product (i.e. TR1). The residual water was further extracted with *n*-butanol. The *n*-butanol fraction was concentrated under vacuum to remove the organic solvent to give a rust-brown product (i.e. TR2), and then TR1 and TR2 were combined to get TR. The residue from the *n*-butanol fraction was further oxidized at pH 8 for 1 h to give a dark product (namely, TB). There were almost no monomeric polyphenols, theaflavins or gallic acid in TR and TB (by HPLC analysis) (data not shown). This showed

that all oxidative procedures of polyphenols were thorough.

2.3. Assay for DPPH radical scavenging activity

The $DPPH^{\cdot -}$ scavenging activity was determined according to the method of Zhu, Robert, Jodi, Roberta, and Carl (2002), with a slight modification. A radical solution, 1 mM of $DPPH^{\cdot}$ in methanol, was prepared, and then 0.8 ml of this solution was mixed with 2.4 ml of test samples dissolved in methanol containing 5–100 μg of the oxidized phenolic compounds; the mixture was then vortexed vigorously and maintained for 30 min at room temperature in the dark. The OD_1 was measured at 517 nm (HITACHI U-3210 spectrophotometer). A control sample containing the same amount of methanol and DPPH radical was prepared and measured at the same wavelength and was recorded as OD_0. The absorbance of samples dissolved in 3.2 ml methanol was recorded as OD_2. This activity is given as $DPPH^{\cdot -}$ scavenging rate and is calculated according to the following equation

$$DPPH^{\cdot -}\text{scavenging rate } (\%) = [OD_0 - (OD_1 - OD_2)] \times 100/OD_0$$

2.4. Chemiluminescence assay for hydroxyl radical scavenging activity

The $OH^{\cdot -}$ scavenging activity was determined by a chemiluminescence method as described by Cheng, Yan, Li, and Chang (2003), with a slight modification. The $OH^{\cdot -}$ was generated by a Fenton-type reaction at room temperature. The reaction mixture (1.0 ml) contained: 600 μl luminol (0.1 mM, diluted in the carbonic acid-buffered saline solution (CBSS), pH 10.2), 100 μl of sample solutions (with different concentration, CBSS replaced the sample in the control), 200 μl Fe^{2+}-EDTA (3 mM) and 100 μl H_2O_2 (1.2 mM). Initiation of reaction was achieved by adding Fe^{2+}-EDTA and then H_2O_2 to the mixture. The chemiluminescence intensity (CL) integral was recorded and the scavenging rate was obtained according to the formula

$$\text{Scavenging rate } (\%) = (\text{CL(control)} - \text{CL(sample)}) \times 100/\text{CL(control)}$$

2.5. Cell culture and treatment

The embryonic human lung fibroblasts (HPF-1), purchased from Peking Union Medical College, were normal diploid fibroblasts. The HPF-1 was maintained in DMEM supplemented with 10% (v/v) heat-inactivated newborn calf serum, 100 IU/ml of penicillin, and 100 μg/ml of streptomycin. Cells were cultured in a humidified atmosphere incubator at 37 °C in 5% CO_2. Culture medium was refreshed every 3 days. Cultures were maintained for 7 days prior to experimentation. Cells were cultured at a density of 2×10^5 cells/ml on 96-well plates and cultured 24 h before treatment. Initially, the culture medium was replaced with fresh medium containing various concentrations of H_2O_2, to determine the optimal oxide damage concentration of H_2O_2 for the following experiments (Koh et al., 2004). The oxidized phenolic compounds were added and maintained for 1 h before treatment with H_2O_2 for a subsequent 24 h.

2.6. MTT assay

Cell viability was measured by quantitative colorimetric assay with MTT, showing the mitochondrial activity of living cells as described in the literature (Denizot & Lang, 1986; Mosmann, 1983). After treatment for 24 h, the medium was removed and fresh medium containing 0.5 mg/ml MTT was added to each well, followed by incubation for 3 h at 37 °C. Finally the medium containing MTT was removed, and cells were lysed with dimethyl sulfoxide (DMSO). The absorbance at 595 nm was measured using a Bio-Rad 3350 microplate reader. Control cells were treated in the same way but without H_2O_2, and the values of different absorbances were expressed as a percentage of control.

2.7. Determination of intracellular reactive oxygen species by DCF-DA

The level of intracellular reactive oxygen species (ROS) was quantified by fluorescence with DCF-DA, as described by Yamamoto et al. (2003). DCF-DA is cell- permeable and non-fluorescent. After it enters into live cells, the diacetate groups are cleaved by intracellular esterases, and DCF-DA is changed into ionized form. Oxidation of the ionized form dyes occurs in the presence of ROS; consequently, the ionized dyes are oxidized to a fluorescent 2′,7′-dichlorofluorescein (DCF), which can be measured by a fluorescent plate reader to reflect levels of intracellular ROS. Thus, values of the fluorescence in the cell cultures are constantly rising in this assay. Cells (1.5×10^4 cells/well) were incubated with Hallam's physiological saline (HPS) containing DCF-DA (10 μM) in a 96-well microplate for 30 min at 37 °C. After the incubation, cells were washed three times with HPS and incubated with HPS containing samples (with different concentrations) for 30 min, then incubated with HPS containing H_2O_2 (600 μM) for the indicated time periods. The intracellular ROS levels were measured by using a fluorescence plate reader (Fluoroskan Ascent 2.4), at an excitation wavelength of 485 nm and an emission wavelength of 538 nm. The measured fluorescence values were expressed as a percentage of the fluorescence in control cells.

2.8. Statistical analysis

All experiments were performed in triplicate. One-way ANOVA was used to estimate overall significance,

followed by *post hoc* Tukey's tests corrected for multiple comparisons (Miller, 1981). Data are presented as means ± SEM. A probability level of 5% ($p < 0.05$) was considered significant.

3. Results and discussion

3.1. Inhibition of hydroxyl radicals and $DPPH^{\cdot -}$

Percent hydroxyl radical scavenging activities of oxidized phenolic compounds were dose-dependent. Quenching ability of oxidized phenolic compounds on the CL signal, which indicated their potentials to scavenge hydroxyl radicals produced in the Fe(II)–H_2O_2–luminol system, was ranked by 50%-inhibition concentrations (IC_{50}). The lower the IC_{50} value, the higher was the activity of oxidized phenolic compounds. From regression analysis of scavenging rate (%) and the natural logarithm of oxidized phenolic compounds concentration, a good linear relationship was observed, and the regression equations and correlation coefficients are listed in Table 1. With the regression equations derived, it was easy to calculate the IC_{50} values of each compound. Comparing the IC_{50} values of each sample (EGCG as a positive control), it appears that TF shows the greatest quenching of hydroxyl radicals, followed by TB and TR. TF acts as a competitive inhibitor and is the most potent inhibitor of hydroxyl radicals produced in the Fe(II)–H_2O_2–luminol system.

Similar to the above experiment on hydroxyl radicals, TF, TR and TB also showed a good linear relationship between scavenging rate (%) and the natural logarithm of oxidised phenolic concentrations in the $DPPH^{\cdot}$ assay. The order of $DPPH^{\cdot -}$ scavenging ability was TF > TB > TR (Table 2), which was similar to the inhibition of hydroxyl radicals (Table 1).

The main sites of catechin antioxidant action appear to be the o-dihydroxy B-ring, or *vic*-trihydroxy B-ring or gallate group through the one electron transfer or H-atom abstraction mechanism (Jovanovic, Hara, Steenken, & Simic, 1995; Nanjo et al., 1996; Sang et al., 2002; Wright, Johnson, & DiLabio, 2001). Theaflavins posses a benzotropolone skeleton that is formed from co-oxidation of appropriate pairs of catechins, one with a *vic*- trihydroxy moiety and the other with an o-dihydroxy structure (Wan, 2003). Therefore, the chemical structures of theaflavins are more bulky and have two A-rings of flavanols linked by a fused seven-member ring. These structural features may provide more interaction sites for radicals, which could partly explain that TF have the most potent antioxidant activity among these oxidised phenolic compounds. Despite the fact that TR is the most abundant of the polyphenolic oxidation products in black tea, the papers concerned with the chemistry of TR at present are limited. Therefore, it is not easy to explain from the chemical structure of TR that TR showed no potent antioxidant activity in this study. To date, there are no definitive data on TB structures. We speculated that some polyphenols in TB (Wan, 2003) make the most contribution to free radical scavenging activity of TB.

Table 1
Quenching ability of oxidized phenolic compounds to quench the chemiluminescence signal caused by hydroxyl radicals[a]

Sample	Regression equation	R^2	Linear range (μg/ml)	IC_{50} (μg/ml)
EGCG[b]	$y = 51.1x + 16.6$	0.988**	0.5–5.0	1.9
TF	$y = 22.3x + 13.1$	0.964**	0.5–25	5.2
TR	$y = 20.9x - 8.9$	0.903*	1.0–50	16.8
TB	$y = 21.5x - 2.5$	0.948**	1.0–50	11.4

[a] y, scavenging rate (%); x, natural logarithm values of corresponding concentrations of oxidized phenolic compounds.
[b] EGCG was used as a control.
* $p < 0.05$.
** $p < 0.01$.

Table 2
$DPPH^{\cdot -}$ scavenging activity of oxidized phenolic compounds[a]

Sample	Regression equation	R^2	Linear range (μg/ml)	IC_{50} (μg/ml)
EGCG[b]	$y = 34.8x - 10.5$	0.998**	2.5–20	5.7
TF	$y = 32.2x - 17.5$	0.967**	2.5–20	8.1
TR	$y = 19.9x - 21.3$	0.895*	2.5–40	36.1
TB	$y = 26.8x - 27.4$	0.964**	2.5–40	18.0

[a] y, scavenging rate (%); x, natural logarithm values of corresponding concentrations of oxidized phenolic compounds.
[b] EGCG was used as a control.
* $p < 0.05$.
** $p < 0.01$.

3.2. Effect on H_2O_2-induced or normal HPF-1 cell viability

The cell viability was expressed as MTT conversion rate. The effects of oxidized phenolic compounds, at different concentrations, on H_2O_2-induced loss of HPF-1 cell viability, are depicted in Fig. 1. Treatment with 600 μM H_2O_2 for 24 h decreased the viability of HPF-1 cells by about 26–32% relative to the negative control. Upon pretreatment with the TF, TR and TB at different concentrations (0.72, 1.43, 2.87, 5.73 and 11.5 μg/ml), the cell viability was almost dose-dependently ameliorated. Oxidized phenolic compounds had a significantly protective effect on H_2O_2-damaged HPF-1 cell ($p < 0.05$), magnitude of the increased viability was about 10% compared with the positive control group. It was equivalent to recovery by almost 30%, of the cell loss. Compared with EGCG, TF and TB showed an inferior protective effect at the concentration of 2.87–11.5 μg/ml. However, at 0.72–1.43 μg/ml, TF and TB was more effective than was EGCG. TR acts as an inferior protector among these oxidized phenolic compounds in this system.

The cell viability result is shown in Fig. 2 when the cells were treated with oxidized phenolic compounds alone. TF and TB caused almost dose-dependent proliferation, whereas the TR result was dose-dependently reduced. TR

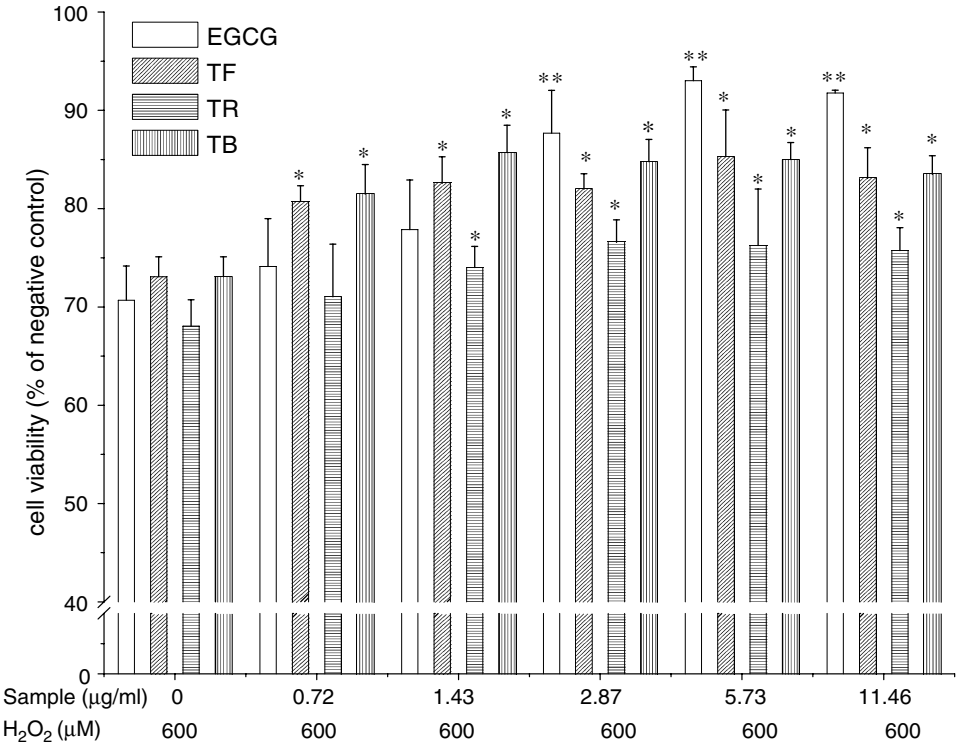

Fig. 1. Effects of oxidized phenolic compounds on H_2O_2-induced decrease of HPF-1 cell viability. Cell viability was estimated by MTT assay after treatment with H_2O_2 (600 μM) and/or oxidized phenolic compounds for 24 h (at indicated concentrations). EGCG was used as a control. Data are expressed as means (OD value) ± S.E.M ($n = 8$), shown by percentage of the negative control (untreated cells); $^{*}p < 0.05$, $^{**}p < 0.01$ when compared with that of the positive control group (treated with only 600 μM H_2O_2).

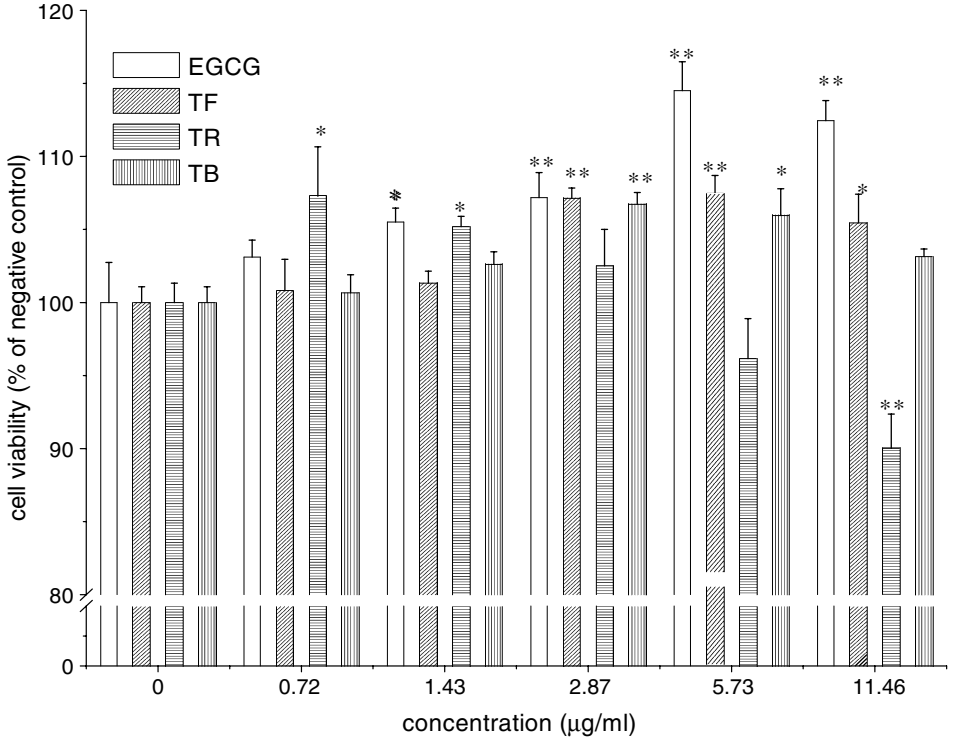

Fig. 2. Effects of oxidized phenolic compounds on normal HPF-1 cell viability. Cell viability was estimated by MTT assay after treatment with oxidized phenolic compounds for 24 h (at indicated concentrations). EGCG was used as a control. Data are expressed as mean (OD value) ± S.E.M ($n = 8$), shown by percentage of the negative control (untreated cells); $^{*}p < 0.05$, $^{**}p < 0.01$ when compared with that of the negative control.

showed a negative effect on normal HPF-1 cells at 11.5 μg/ml ($p < 0.01$).

HPF-1 cells, human normal diploid fibroblasts, exhibit finite proliferative potential *in vitro*, the so-called Hayflick limit (Hayflick, 2000). They undergo a limited number of population doublings before entering a state of permanent growth arrest, referred to as "replicative senescence," "cellular senescence" or "cellular aging" (Campisi, 1996), in which they remain alive and metabolically active but are completely refractory to mitogenic stimuli. HPF-1 offers a typical model for studying the process of aging *in vitro*. Various oxidative stresses have been used to study the onset of cellular senescence. The early onset of cellular senescence, induced by oxidative stresses, is termed as stress-induced premature senescence (SIPS) (Toussaint, Medrano, & von Zglinicki, 2000), and H_2O_2 has been the most commonly used inducer of SIPS (Frippiat, Dewelle, Remacle, & Toussaint, 2002), which shares features of replicative senescence: similar morphology, senescence-associated ß-galactosidase activity, cell cycle regulation (Chen & Ames, 1994; Dimri et al., 1995).

3.3. Effecst on the accu mulation of ROS in H_2O_2-induced or normal HPF-1 cell

ROS are the main cause of oxidative stress, which results in decreasing of cell viability. The level of DCF fluorescence is an indicator of ROS production. As shown in the Fig. 3, 600 μM H_2O_2 was able to produce these toxic species in the HPF-1 cells; exposure to H_2O_2 for 1 h increased the DCF fluorescence intensity by about 60–70% over the negative control. The increase in the DCF fluorescence intensity was partly eliminated when the cells were co-treated with different concentrations of EGCG, TF, TR and TB. The decrease of fluorescence intensity was almost dose-dependent. At the concentration of 0.96–3.84 μg/ml, TF and TB showed stronger ability to eliminate the fluorescence intensity than did EGCG. In addition, with no exposure to H_2O_2, the DCF fluorescence intensity in the HPF-1cells, after the cells were treated with oxidized phenolic compounds at the concentration of 0.96–3.84 μg/ml for 1 h, also showed some decrease. These results implicate the involvement of ROS in H_2O_2-induced

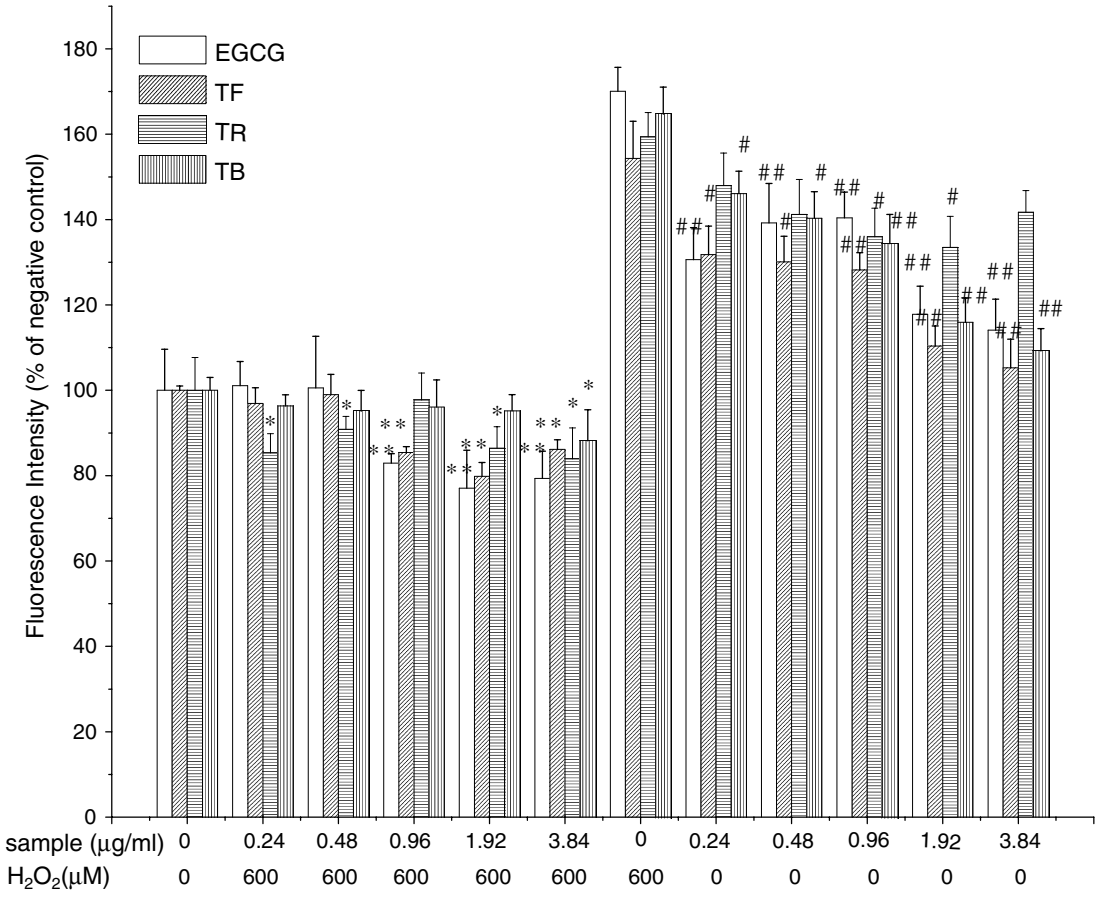

Fig. 3. Effects on the accumulation of ROS in H_2O_2-induced or normal HPF-1 cells by oxidized phenolic compounds. The fluorescence intensity of DCF was measured after HPF-1 cells were exposed to oxidized phenolic compounds (indicated concentration) and/ or H_2O_2 (600 μM) for 1 h. Data are expressed as mean (fluorescence intensity value) ± S.E.M ($n = 8$), shown by percentage of the negative control (untreated cells); $^{*}p < 0.05$, $^{**}p < 0.01$ compared with that of the negative control (untreated cells); $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with that of the positive control (treated with only H_2O_2).

loss of cell viability and the antioxidant effect of oxidized phenolic compounds in HPF-1 cells.

It is well known that the generation of ROS is associated with the initiation and promotion of carcinogenesis. Excessive amounts of ROS increase oxidative stress in the body. In recent years, many studies have suggested that green tea polyphenols (catechins) are good scavengers of ROS *in vitro* (Hu et al., 1995; Nanjo, Mori, Goto, & Hara, 1999). TF (monomer), as an oxidized phenolic product, proved more effective than did EGCG in suppression of intracellular reactive oxygen species in HL-60cells (Lin et al., 2000). Some studies have indicated that TR protects against oxidative DNA damage in*vivo*, by scavenging ROS (Giovannelli et al., 2000; Halder & Bhaduri, 1998; Lodovici et al., 2000). TR was usually regarded as ''tea pigments or black tea extract" in these reports. Bioavailability of thearubigins as oxidative phenol compounds has not so far been described. Unlike the well-examined and published antioxidant properties of TF, bioactivity of TB has hardly been discussed. TB has attracted little interest so far due to the complexity of its ingredients and its negative effect on black tea quality.

4. Conclusion

In the present study, we have extended studies to hydroxyl radicals and DPPH· inhibition (Tables 1 and 2) and effect on the H_2O_2-induced oxidation or normal HPF-1 cells (Figs. 1 and 2) and scavenging of ROS in H_2O_2-affected or normal HPF-1 cells (Fig. 3). It appears that TF shows the most antioxidant effect in the above four systems (among these oxidized phenolic compounds). However, TR acted as a weak antioxidant. Interestingly, TB, as an oxidised product from TF or TR, showed potent antioxidant ability, which closely followed TF. These oxidized phenolic compounds protected against oxidative damage in HPF-1 cells, by suppressing intracellular reactive oxygen species in the cells.

TF, TR and TB, as the major oxidized phenolic products during the fermentation stage of black tea processing, have the most effect on the quality and make the most contribution to the antioxidant activity of black tea. In this study, TB behaviour is outstanding. There is a need for more detailed studies to enhance the harmony between quality and bioactivity in the black tea process.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2007.05.006.

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