Journal of Medicinal Plants Research Vol. 6(1), pp. 114-121, 9 January, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1308
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Free radical scavenging abilities *in vitro* and antioxidant activities *in vivo* of black tea and its main polyphenols

Shili Sun, Shunshun Pan, Caijin Ling, Aiqing Miao, Shi Pang, Zhaoxiang Lai, Dong Chen and Chaoyi Zhao*

Tea Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, P. R. China.

Accepted 21 October, 2011

In the present study, *in vitro* free radical scavenging abilities and *in vivo* antioxidant activities of Assam black tea extract (ASTE) and theaflavins mixture (TFSM) were investigated. Our results showed that ASTE and TFSM had significant free radical scavenging activities *in vitro* in a dose-dependent manner, especially 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical. For antioxidant activities *in vivo*, the mice were fed with ASTE and TFSM everyday. The contents of malondialdehyde (MDA), and the activities of superoxide dismutase (SOD) and glutathione per- oxidase (GSH-Px) in the mice serum and liver were measured after 30 days. As a result, TFSM could obviously improve SOD and GSH-Px activities, and reduce MDA content in the serum and liver. Though ASTE also showed a certain antioxidant activity, it was slightly weaker than the antioxidant activity of TFSM in the mice. Moreover, ASTE did not change the activity of GSH-Px in the mice liver. In conclusion, our results showed that black tea and its main polyphenols were effective in scavenging DPPH, ABTS and OH free radicals *in vitro*, and increasing the SOD and GSH-Px activities, decreasing the MDA contents in the mice. These findings suggest the important significance of black tea consumption in prevention of diseases.

Key words: Black tea, polyphenols, antioxidant, theaflavin.

INTRODUCTION

Teas are produced from the leaves of *Camellia sinensis* (L.) O. Kuntza (Theaceae), and usually classified according to the manufacturing process into three categories of fermented (black), unfermented (green), and semifermented (oolong). As shown in Figure 1A, the polyphenols in green tea include (-)-epigallocatechin gallate (EGCG), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), (-)-epicatechin (EC), (+)-catechin (C), (-)-gallocatechin (GC), (-)-catechin gallate (CG), and (-)-gallocatechin gallate (GCG) (Murakami et al., 2006). In black tea, crushed and withered tea leaves are allowed to undergo oxidative fermentations, leading to the formation of oligomers, such as theaflavins and polymers, known as thearubigins. In black tea, the aforementioned catechins are reduced to about one-tenth of those in green tea and theaflavins account for about 1 to 2% of the total dry matter. The theaflavins, including theaflavin (TF1), theaflavin-3-gallate (TF2A), theaflavin-3'-gallate (TF2B), theaflavin-3, and 3'-digallate (TF3) (Figure 1B), are responsible for characteristic taste, fragrance and color of black tea (Babich et al., 2006). The other major components of black tea infusions are thearubigins, which are heterogeneous mixtures of catechin oxidation products and have not yet been chemically characterized. In addition, there are many uncharacterized colorless polyphenolic compounds derived from green tea catechins. These chemically unknown substances may be partly produced due to further oxidation of theaflavins as described earlier (Takashi and Isao, 2003).

Nowadays, green tea is considered one of the most promising dietary agents for the prevention and treatment of many diseases because numerous studies have demonstrated that aqueous extract of the major green tea

***Corresponding author. E-mail: zhaochaoyi@sogou.com. Tel: +86-020-8516-1054. Fax: +86-020-8516-1454.

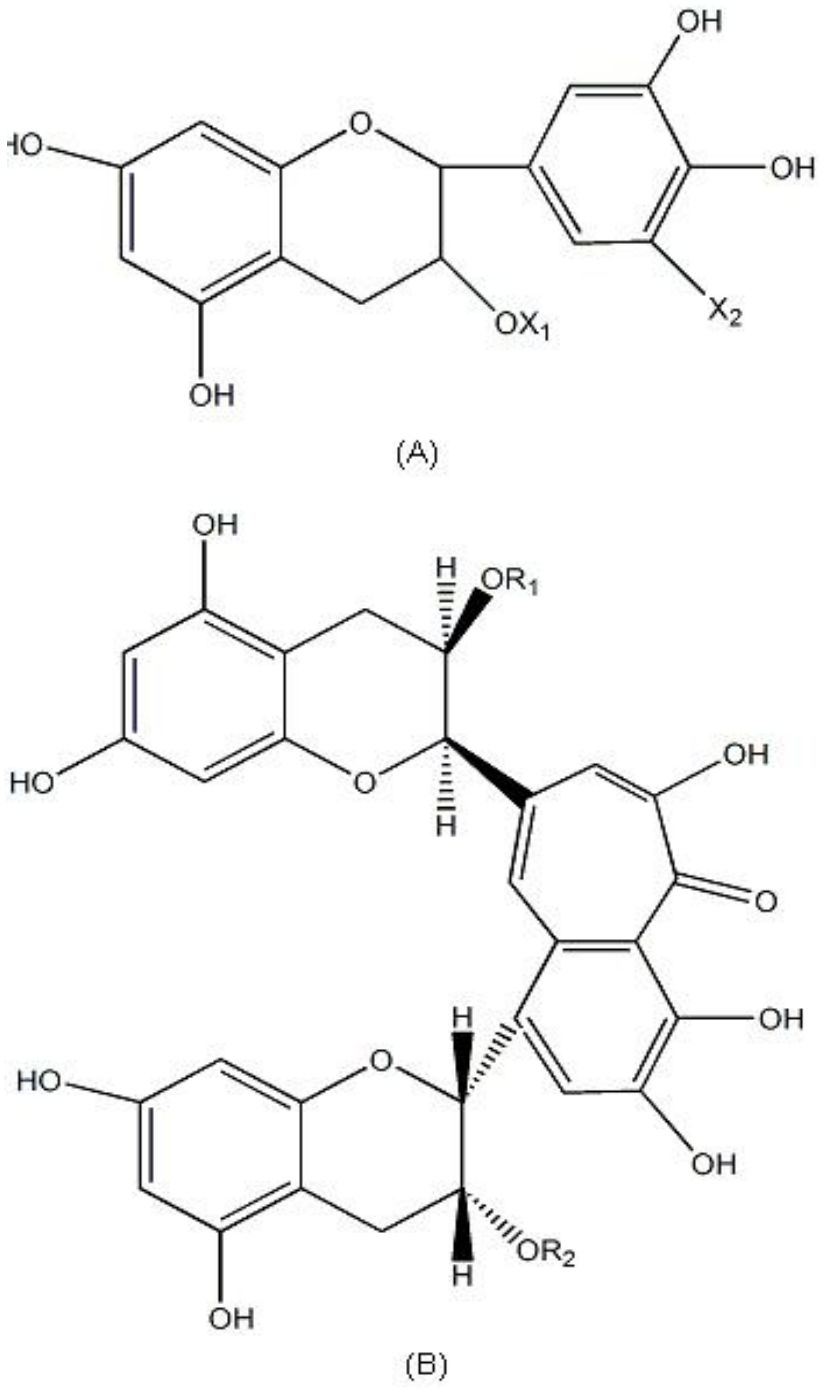

Figure 1. Chemical structures of catechins (A) and theaflavins (B). L-EC, D-C ($X_1 = X_2 = H$); L-EGC, D-GC ($X_1 = H$, $X_2 = OH$); L-ECG, D-CG (X_1 = galloyl, $X_2 = H$); L-EGCG, D-GCG (X_1 = galloyl, $X_2 = OH$); TF1 = Theaflavin ($R_1 = R_2 = H$); TF2A = Theaflavin-3-gallate (R_1 = galloyl, $R_2 = H$); TF2B = Theaflavin-3'-gallate ($R_1 = H$ R_2 = galloyl); TF3 = Theaflavin-3,3'-digallate ($R_1 = R_2$ = galloyl).

polyphenols designed as catechins possess antioxidant, anti-mutagenic, anti-diabetic, anti-inflammatory, anti-bacterial and anti-viral, and above all, cancer-preventive properties (Cabrera et al., 2006). Black tea is consumed in some Asian countries and Western nations and accounts for 80% of total tea consumed (Sharma and Rao, 2009). Therefore, in recent years, black tea is extensively investigated. However, studies on biological

activities of black tea are far behind those of green tea. Black tea has been regarded due to its low content of monomeric polyphenols, as having significantly weaker antioxidative properties than that of green tea. As a matter of fact, partial polymerization and other alterations occurring during fermentation of tea leaves did not diminish the antioxidative properties of black tea. It was likely that other unidentified constituents of black tea contribute to its beneficial role (Halder and Bhaduri, 1998).

The purpose of the present study was to examine the antioxidant activities of black tea *in vitro* and *in vivo*. The free radical scavenging abilities by the extracts of black tea and its main polyphenols were investigated. The contents of MDA, the activities of SOD and GSH-Px in the mice serum and liver were also determined.

MATERIALS AND METHODS

Chemicals

2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical were purchased from Sigma (Saint Louis, USA). Assay kits for malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjiang, China). Assam black tea was purchased from Premier's Tea Limited in India. Theaflavins mixtures (TFSM, 50% purity) were obtained from Hangzhou Easily Biotechnology Co. Ltd (Hangzhou, China). All other chemicals used in the study were extra-pure grade or analytical grade.

Preparation of black tea extract and determination of its polyphenols

Assam black tea was minced and extracted three times through placing in the boiling distilled water for 10 min each time (tea/water, 1:5 w/v). These extracted solutions were combined and concentrated at 60°C with RE-2000 Rotary Evaporator (Yarong Biochemical Instrument, Shanghai, China). Finally, the solution was dried by lyophilization (Savant Novalyphe-NL500, USA) to obtain the aqueous extract of Assam black tea (ASTE).

The contents of catechin and theaflavin monomers were analyzed using a high performance liquid chromatography (HPLC) system (Shimadzu LC-2010, Kyoto, Japan) (Tu et al., 2005). The ASTE and TFSM were analyzed on a Diamonsil C18 column (4.6×250 mm, 5 µm particle size, Japan). The eluate was monitored at 280 nm. Mobile phase A and B were made of acetic acid/acetonitrile/water (A: 0.5:3:96.5 and B: 0.5:30:69.5, by vol.). The flow rate was set at 1 ml/min and 10 µl sample was injected into the column. The elution was performed using a linear gradient from solvent A to solvent B in 45 min followed by an isocratic step of solvent B for 15 min. The column temperature was set to 28°C. Peaks were identified in comparison with the retention time of authentic standards.

Scavenging ability on DPPH free radical

The antioxidant abilities of ASTE were determined by measuring the capacity of bleaching a purple colored ethanol solution of DPPH as described by Turkoglu et al. (2007) with slight modification. Briefly, various concentrations samples (3 ml) in ethanol were added to 1 ml of a 0.1 mmol/L DPPH in ethanol. The mixture was then vortexed vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm. The DPPH free radical scavenging rate was calculated in the following formulation:

% DPPH scavenging = (control absorbance - sample absorbance) × 100% /control absorbance.

Scavenging ability on ABTS free radical

ABTS scavenging assay was carried out following a modified method (Thana et al., 2008). Briefly, ABTS radical cation (ABTS) solution was produced by reacting ABTS stock solution with 2.45 mmol/L potassium persulfate in the dark for 12 h and adjusting the absorbance to 0.70±0.02 at 734 nm. For the photometric assay, 3 ml of the ABTS solution and 100 µL samples solution were mixed and measured immediately after 6 min at 734 nm (absorbance did not change significantly up to 10 min). The ABTS free radical scavenging rate was calculated in the following formulation:

% ABTS scavenging = (control absorbance - sample absorbance) × 100% /control absorbance.

Scavenging ability on OH free radical

$FeSO_4$, H_2O_2 and yeast were dissolved in citric acid buffers (pH 5.6). 0.2 ml H_2O_2 (5%), 0.2 ml yeast solution (75 mg/ml), 0.4 ml various concentrations samples and 0.4 ml $FeSO_4$ (0.8 mmol/L) were mixed according to the order in tube. The chemiluminescence intensity (CLI) was measured by Luminescence measurement (Lumat LB 9507, Germany). CLI was simultaneously recorded once per 3 s (citric acid buffers replaced the sample in the control). The scavenging rate was obtained according to the following formula:

% OH scavenging = [CLI (control) - CLI (sample)] × 100%/ CLI (control)

Animals and experimental design

A total of 70 male Kunming mice (18 to 22 g in weight) were obtained from Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China), and were maintained in an air conditioned room (25±1°C) with a 12 h light/dark cycle. They were fed with the standard laboratory diet and given water. The experimental protocol was reviewed and approved by the Zhejiang University Animal Care Committee in accordance with the “Guide for the Care and Use of Laboratory Animals”. Animals were randomly divided into seven groups consisting of ten mice each: normal control group, feeding ASTE groups (low, moderate and high dose group), feeding TFSM groups (low, moderate and high dose group). Three dose groups were, respectively, fed with ASTE or TFSM in three different doses: 0.5, 1.0 and 2.0 g/kg body weight per day treated to the mice by gavage for 30 days. Normal control group was given only double distilled water by oral gavage for 30 days.

After 30 days, the animals were sacrificed by decapitation. Blood samples were centrifuged at 4000 g at 4°C for 5 min. The activities of SOD and GSH-Px and MDA contents in the serum were measured according to the instructions of the kits. The liver was excised, weighed and homogenized in 0.1 g/ml of ice-cold isotonic physiological saline based on wet weight. The suspension was centrifuged at 4000 g at 4°C for 10 min; the supernatant was subjected to the measurement of the activities of SOD and GSH-Px and MDA contents in the liver according to the instructions of the kits.

Table 1. Catechin and theaflavin monomers contents of Assam black tea extract (ASTE) and theaflavins mixture (TFSM) as analyzed by HPLC (mg/g).

Sample	C	EC	GC	EGC	CG	ECG	GCG	EGCG	TF1	TF2A/TF2B	TF3
TFSM	15.66	32.01	140.35	30.23	1.97	22.33	5.58	9.68	199.08	118.89	193.34
ASTE	17.36	43.15	192.52	88.46	3.91	22.64	6.22	19.92	5.80	6.40	8.20

Statistical analysis

Results were expressed as mean ± SD of at least three independent replications of each experiment. Statistical significance was determined by pair t-test analysis using Origin 7.5 software for windows. Mean values were considered significant difference at $p < 0.05$ or $p < 0.01$.

RESULTS

Catechin and theaflavin monomers contents

Catechin and theaflavin monomers contents of ASTE were analyzed by HPLC. As shown in Table 1, eight kinds of catechin monomer contents of ASTE were respectively higher in comparison with those of the control TFSM. Meanwhile four kinds of theaflavin monomer contents of TFSM were respectively far higher than those of ASTE.

Free radical scavenging abilities *in vitro*

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating the free radical scavenging activities of antioxidants (Hu et al., 2004). As shown in Figure 2A, DPPH free radical scavenging rate of ASTE and TFSM increased significantly in a dose-dependent manner. At 1.25 and 2.5 μg/ml concentrations, the DPPH free radical scavenging ability of ASTE was better than that of TFSM. However, at other treatment concentrations, the DPPH free radical scavenging ability of TFSM was stronger than that of ASTE.

ABTS free radical is another synthetic radical and more versatile than DPPH, and the ABTS model can be used to assess the scavenging activity for both the polar and non-polar samples (Re et al., 1999). From the results (Figure 2B), ASTE and TFSM were able to scavenge ABTS free radical in a dose-dependent manner. At 1.25 and 5 μg/ml concentrations, ABTS free radical scavenging ability of ASTE was better than that of TFSM. However, at 2.5 and 10 μg/ml concentrations, ABTS free radical scavenging ability of TFSM was stronger than that of ASTE. It is worth noting that the scavenging rate of ASTE and TFSM were both >96% at a dosage of 20 μg/ml.

Hydroxyl free radical and its subsequent radicals are the most harmful reactive oxygen species (ROS) and are mainly responsible for the oxidative injury of biomolecules. The OH free radical scavenging data (Figure 2C) indicated that ASTE and TFSM were also able to scavenge OH free radical in a dose-dependent manner. At all treatment concentrations, OH free radical scavenging ability of ASTE was weaker than that of TFSM. Moreover, OH free radical scavenging rate of ASTE and TFSM treatments were no more than 50%. That was to say, ASTE and TFSM did possess the ability to scavenge OH free radical, but they were less efficient than the DPPH and ABTS free radical.

SOD, GSH-Px activities and MDA contents in the mice serum

Table 2 showed the SOD and GSH-Px activities as well as MDA contents in the serums of the mice. A significant increase of antioxidant enzymes activities (SOD and GSH-Px) ($p < 0.01$) and decrease of MDA contents ($p < 0.01$) were observed in the TFSM treatment groups in comparison with the normal control group. In addition, the SOD and GSH-Px activities increased with the concentration of TFSM treatment, and MDA contents decreased with TFSM treatment concentration. But SOD activities were not changed significantly by ASTE treatment except at 1.0 g/kg·BW treatment. Meantime, a significant increase of GSH-Px activities ($p < 0.01$) and decrease of MDA contents ($p < 0.01$) except at 1.0 g/kg·BW treatment were also observed in the ASTE treatment groups compared to the normal control group.

SOD, GSH-Px activities and MDA contents in mice liver

The SOD, GSH-Px activities and MDA contents in livers of the mice were shown in Table 3. A significant increase of GSH-Px activities ($p < 0.01$) and decrease of MDA contents ($p < 0.01$) were showed in the TFSM treatment groups in comparison with the normal control group. The SOD activities significantly increased ($p < 0.05$ or $p < 0.01$) at the dose of 0.5 and 1.0 g/kg·BW, but there were no significant difference ($p < 0.05$) between the normal control group and high dose group (2.0 g/kg·BW). In addition, a significant increase of SOD activities ($p < 0.05$ or $p < 0.01$) and decrease of MDA contents ($p < 0.05$) were observed in the ASTE treatment groups compared to the normal control group. However, GSH-Px activities were not significantly changed by ASTE treatment.

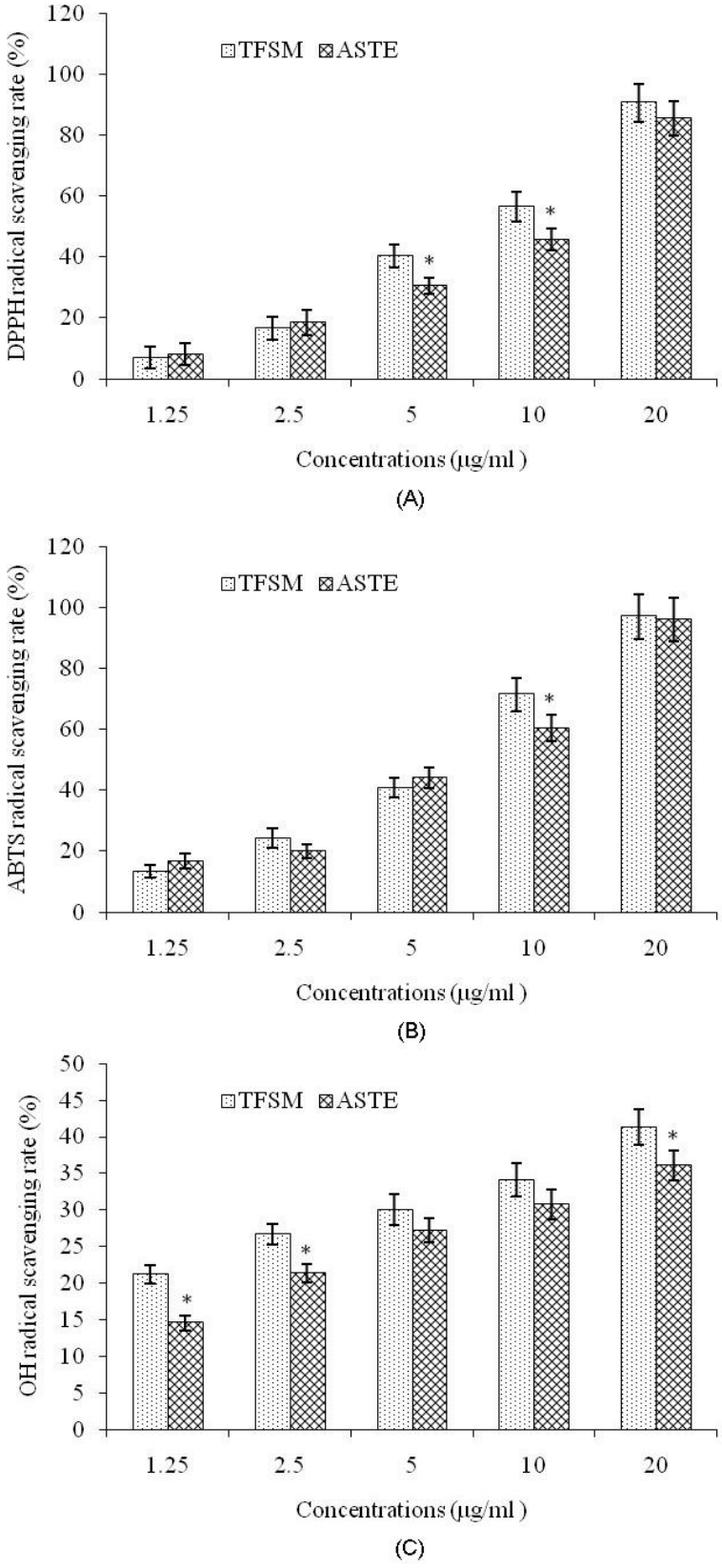

Figure 2. Scavenging abilities of Assam black tea extract (ASTE) and theaflavins mixture (TFSM) on DPPH free radical (A), ABTS free radical (B) and OH free radical (C). Each value represents the mean ± SD (n=3). * $p < 0.05$ compared with TFSM.

Table 2. The effects of Assam black tea extract (ASTE) and theaflavins mixture (TFSM) on the activities of SOD (U/ml) and GSH-Px (U) and MDA (nmol/ml) contents in the serum of mice.

Group	SOD activity	GSH-Px activity	MDA contents
Normal control group	53.71±20.13	138.55±8.39	13.87±0.93
TFSM (0.5 g/kg·BW)	482.94±15.25[b]	162.71±6.78[b]	9.07±0.96[b]
ASTE (0.5 g/kg·BW)	88.80±23.33	181.40±12.36[b]	8.98±0.84[b]
TFSM (1.0 g/kg·BW)	516.02±14.03[b]	169.38±3.79[b]	9.08±0.82[b]
ASTE (1.0 g/kg·BW)	102.70±25.09[a]	173.70±9.88[b]	12.70±1.01
TFSM (2.0 g/kg·BW)	521.21±13.65[b]	170.00±11.22[b]	4.93±0.12[b]
ASTE (2.0 g/kg·BW)	72.90±20.05	193.98±13.63[b]	9.10±0.87[b]

Data were presented as means ± SD (n = 10). [a] $p < 0.05$, compared with normal control group and [b] $p < 0.01$, compared with normal control group.

Table 3. The effects of Assam black tea extract (ASTE) and theaflavins mixture (TFSM) on the activities of SOD (U/mg protein) and GSH-Px (U/g protein) and MDA (nmol/mg protein) contents in the liver of mice.

Group	SOD activity	GSH-Px activity	MDA contents
Normal control group	12.02±0.30	134.62±8.41	3.52±0.47
TFSM (0.5 g/kg·BW)	14.05±0.49[a]	480.18±22.15[b]	1.26±0.11[b]
ASTE (0.5 g/kg·BW)	13.94±0.61[a]	118.80±7.22	2.59±0.42[a]
TFSM (1.0 g/kg·BW)	16.15±0.94[b]	486.49±64.71[b]	0.74±0.06[b]
ASTE (1.0 g/kg·BW)	17.10±1.21[b]	147.60±10.06	3.00±0.49[a]
TFSM (2.0 g/kg·BW)	9.20±4.87	637.06±32.98[b]	1.59±0.06[b]
ASTE (2.0 g/kg·BW)	15.50±6.88[b]	134.10±9.98	2.46±0.40[a]

Data were presented as means ± SD (n = 10). [a] $p < 0.05$ compared with normal control group and [b] $p < 0.01$, compared with normal control group.

DISCUSSION

The tea is the most widely used ancient beverage in the world. Green tea is primarily consumed in some Asian countries, such as Japan, China, Korea, and India, and a few countries in North Africa and the Middle East. Black tea is consumed in some Asian countries and Western nations (Sharma and Rao, 2009). Biological activity of green tea has been extensively studied, and 80% of the approximately 2.5 million metric tons of manufactured dried tea is black tea. Therefore, in recent years, black tea is extensively investigated mainly regarding its influence on human health (Rietveld and Wiseman, 2003). Certain bioactive compounds present in black tea possess antioxidant properties. Tea drinking improves the antioxidant status *in vivo* and lowers the risk of health problems such as some types of cancers, mutations, coronary heart diseases, stroke, and others (Luximon-Ramma et al., 2005). Polyphenols present in black tea are responsible for its antioxidant activity. Catechins, theaflavins, and gallic acid contribute to the antioxidant characteristics of tea (Vinson and Dabbagh, 1999).

In the present study, we showed that Assam black tea extract and theaflavins mixture have scavenging effects on free radical *in vitro*, especially DPPH and ABTS (Figure 2). Theaflavin monomers present in Assam black tea and theaflavins mixture could exert an important antioxidant effect, which was consistent with previous studies (Yang et al., 2008; Miller et al., 1996). In fact, 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 ortho-dihydroxy structure (Sarkar and Bhaduri, 2001). The chemical structures of theaflavins including TF1, TF2A/TF2B and TF3 have two A-rings of flavanols linked by a fused seven-member ring. These structural features can provide more interaction sites with radicals.

Black tea also shows a positive role in clearing other radicals. For example, black tea provides protection against reactive oxygen and nitrogen species. It was found to inhibit the production of compounds like NO and superoxide in murine peritoneal macrophages. Theaflavins were the most effective compounds in down-regulating nitric oxide synthase and black tea was also found to be a better chemo-preventer than green tea (Thiagarajan et al., 2001). Aqueous tea extracts can also quench reactive oxygen species such as singlet oxygen, superoxide, and hydroxyl radicals, prevent oxidative cross linking of proteins and inhibit single strand breakage of DNA in cells. Oxidative stress due to

cigarette smoking was also found to be reduced by black tea. Administration of tea (green/black) extracts can prevent cataract formation and retard the progression of lens opacity (Luczaj and Skrzydlewska, 2004). Alcohol intake causes oxidative imbalance in cells. During metabolism of ethanol, free radicals, and superoxide and hydrogen peroxide radicals are generated. Ethanol induces oxidative stress and also depletes the antioxidant defense system. Since antioxidant mechanisms of body can no longer protect against the reactive compounds, severe damage to cell components may take place. Black tea, which provides polyphenol antioxidants, theaflavins and catechins, was found to protect against this alcohol-induced damage in the mice. The antioxidant effects of black tea were found to be the strongest in the liver, and theaflavins were found to be the most active constituents (Saha and Das, 2003).

Because of the complexity of an *in vivo* system, it is difficult to extrapolate *in vitro* data to the intact organism. Serafini et al. (2000) compared the antioxidant activity of phenol rich beverages including black tea, green tea, and alcohol-free red wine. The results of *in vitro* and *in vivo* studies on antioxidant activity of these substances vary a lot. *In vitro* studies indicate that all polyphenol have high antioxidant activity and can prevent low density lipoprotein (LDL) oxidation. The antioxidant capacity of red wine was found to have higher activity against LDL oxidation than teas. Theaflavins and thearubigins present in black tea show a lower antioxidant activity *in vitro* than green tea phenolics. The *in vivo* activity of black tea was found to be unexpectedly higher than green tea as well as wine. Higher activity of black tea *in vivo* may be due to changes in the structure of polyphenols during digestion and assimilation.

Antioxidative properties of black tea are manifested by its influence on increasing the activity/ concentration of antioxidants in the organism. Water infusion/ crude extract of tea was also found to stimulate the activity of glutathione -S transferase, glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT). Theaflavin was the main activity-modulating component. The activation of these enzymes decreased the lipid peroxidation considerably (Saha and Das, 2003). A 2-month long ingestion of standard food containing dried leaves of black tea by rats protected the liver against decrease in GSH content resulting of carbon tetrachloride activity (Sur-Altiner and Yenice, 2000). In other experiments, mice exposed to the carcinogen, 3-methylcolanthrene showed enhancement of serum superoxide dismutase (SOD) activity as a result of oral administration of black tea solution (Das et al., 2002). From our results (Tables 2 and 3), theaflavins mixture showed significant increase of antioxidant enzymes activities (SOD and GSH-Px) and decrease of MDA contents in the serum and liver of the mice. Though Assam black tea also showed a certain antioxidant activity, it was slightly weaker than that of theaflavins mixture in the mice. Unfortunately, Assam black tea did not change the activities of GSH-Px in the mice liver. These inconsistent findings may be due to the difference of the experimental materials, as well as the dose and structures of antioxidants.

Black tea represents a major source of dietary polyphenols among regular tea drinkers. However, the major oxidation products with larger molecular size, the so-called thearubigins, are still largely unknown, despite their importance in the biological activities of black tea polyphenols. Moreover, whether or not the concentrations at which the bioactive components are present in tea brew and the amount of tea normally consumed are sufficient for the effects on health to show up is not certain. So, whether the results of *in vitro* experiments are meaningful in the context of *in vivo* situation needs to be investigated further.

In conclusion, our data showed that black tea and its main polyphenols were effective in scavenging DPPH, ABTS and OH free radicals *in vitro*, increasing the SOD and GSH-Px activities, and decreasing the MDA contents in the mice. These findings suggest the important significance of black tea consumption in prevention of diseases.

ACKNOWLEDGEMENTS

This project was supported by president foundation of Guangdong Academy of Agricultural Sciences (No. 201018), Guangdong Provincial Key Laboratory of Tea Plant Resources Innovation and Utilization (No. 2008A060301004), and Tea Industry Technology System of China (No. CARS-23).

REFERENCES

Babich H, Pinsky SM, Muskin ET, Zuckerbraun HL (2006). *In vitro* cytotoxicity of a theaflavin mixture from black tea to malignant, immortalized, and normal cells from the human oral cavity. Toxicol. *In vitro*, 20: 677-688.

Cabrera C, Artacho R, Gimenez R (2006). Beneficial effects of green tea--a review. J. Am. Coll. Nutr., 25: 79-99.

Das M, Chaudhuri T, Goswami SK, Murmu N, Gomes A, Mitra S, Besra SE, Sur P, Vedasiromoni JR (2002). Studies with black tea and its constituents on leukemic cells and cell lines. J. Exp. Clin. Cancer Res., 21: 563-568.

Halder J, Bhaduri AN (1998). Protective role of black tea against oxidative damage of human red blood cells. Biochem. Biophys. Res. Commun., 244: 903-907.

Hu F, Lu R, Huang B, Liang M (2004). Free radical scavenging activity of extracts prepared from fresh leaves of selected Chinese medicinal plants. Fitoterapia, 75: 14-23.

Luczaj W, Skrzydlewska E (2004). Antioxidant properties of black tea in alcohol intoxication. Food Chem. Toxicol., 42: 2045-2051.

Luximon-Ramma A, Bahorun T, Crozier A, Zbarsky V, Datla KP, Dexter DT, Aruomad OI (2005). Characterization of the antioxidant functions of flavonoids and proanthocyanidins in Mauritian black teas. Food Res. Int., 38: 357-367.

Miller NJ, Castelluccio C, Tijburg L, Rice-Evans C (1996). The antioxidant properties of theaflavins and their gallate esters-radical scavengers or metal chelators? FEBS Lett., 392: 40-44.

Murakami I, Nakamura T, Ishibashi Y, Shibuya R, Ayano E, Morita-Murase Y, Nagata Y, Kanazawa H (2006). Simultaneous

determination of catechins and procyanidins in bottled tea drinks by LC/MS. Chromatography, 27: 27-33.
Re R, Pellegrinni N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Radic. Biol. Med., 26: 1231-1237.
Rietveld A, Wiseman S (2003). Antioxidant effects of tea: evidence from human clinical trials. J. Nutr., 133: 3285-3292.
Saha P, Das S (2003). Regulation of hazardous exposure by protective exposure: modulation of phase II detoxification and lipid peroxidation by Camellia sinensis and Swertia chirata. Teratog. Carcinog. Mutagen, 1: 313-322.
Sarkar A, Bhaduri A (2001). Black tea is a powerful chemopreventor of reactive oxygen and nitrogen species: comparison with its individual catechin constituents and green tea. Biochem. Biophys. Res. Commun., 284: 173-178.
Serafini M, Laranjinha JA, Almeida LM, Maiani G (2000). Inhibition of human LDL lipid peroxidation by phenol-rich beverages and their impact on plasma total antioxidant capacity in humans. J. Nutr. Biochem., 11: 585-590.
Sharma V, Rao LJ (2009). A thought on the biological activities of black tea. Crit. Rev. Food Sci. Nutr., 49: 379-404.
Sur-Altiner D, Yenice B (2000). Effect of black tea on lipid peroxide and glutathione levels in female rats. Drug Metabol. Drug Interact., 16: 299-305.
Takashi T, Isao K (2003). Oxidation of Tea Catechins: Chemical Structures and Reaction Mechanism. Food Sci. Technol. Res., 9: 128-133.
Thana P, Machmudah S, Goto M, Sasaki M, Pavasant P, Shotipruk A (2008). Response surface methodology to supercritical carbon dioxide extraction of astaxanthin from Haematococcus pluvialis. Bioresour. Technol., 99: 3110-3115.
Thiagarajan G, Chandani S, Sundari CS, Rao SH, Kulkarni AV, Balasubramanian D (2001). Antioxidant properties of green and black tea, and their potential ability to retard the progression of eye lens cataract. Exp. Eye Res., 73: 393-401.
Tu YY, Xia HL, Watanabe N (2005). The changes in catechins during the fermentation of green tea. Prikl. Biokhim. Mikrobiol., 41: 652-655.
Turkoglu A, Duru ME, Mercan N, Kivrak I, Gezer K (2007). Antioxidant and antimicrobial activities of Laetiporus sulphureus (Bull.) Murrill. Food Chem., 101: 267-273.
Vinson JA, Dabbagh YA (1999). Tea phenols: antioxidant effectiveness of teas, tea components, tea fractions and their binding with lipoproteins. Nutr. Res., 18: 1067-1075.
Yang Z, Jie G, Dong F, Xu Y, Watanabe N, Tu Y (2008). Radical-scavenging abilities and antioxidant properties of theaflavins and their gallate esters in H_2O_2-mediated oxidative damage system in the HPF-1 cells. Toxicol. *In vitro*, 22: 1250-1256.