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Full Length Research Paper

Antioxidant activity of bamboo-leaf extracts from the species *Dendrocalamopsis oldhami*

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The antioxidant enzymatic activities of *Dendrocalamopsis oldhami* bamboo-leaf extracts (BLE) in cholesterol-fed rats and an index of lipid peroxidation were determined. In the BLE groups, compared with the control group, significant decrease was observed in superoxide dismutase (SOD) activity in the livers and kidneys, catalase (CAT) activity in the kidneys, and glutathione peroxidase (GSH-Px) activity in the liver and kidneys. Also, significant decrease was observed in the quantities of thiobarbituric acid reactive substances (TBARS) in the liver and kidneys of the BLE groups. A battery of test methods was used to assess the *in vitro* antioxidant activities of the extracts. The half maximal inhibitory concentration (IC_{50}) value was found to be 2.36 mg/g by determining the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities. The total antioxidant activity was 84 mg vitamin C equivalent in per gram BLE (mg VCE/g BLE), and the reducing power was 74 mg quercetin equivalent in per gram BLE (mg QE/g BLE). It was concluded that sugars, proteins, and flavonoids, which made up 18, 16, and 3.7% of the extract, respectively, could have contributed to the production of the antioxidant benefits.

Key words: Bamboo-leaf extracts**,** antioxidant activity**,** flavonoids**,** catalase (CAT) activity**,** glutathione peroxidase (GSH-Px) activity**,** superoxide dismutase (SOD) activity.

INTRODUCTION

Bamboo is one of the most valuable, naturally-occurring plants worldwide because its different edible parts contain important nutrients. Bamboo leaves have been used in traditional Chinese medicine for treating fever and for detoxification for over 1000 years (Zhang and Ding, 1996). In addition, the antioxidants contained in bamboo leaves have been included in China's National Standards (GB-2760) as a kind of food antioxidant. Published research data show different pharmacological activities of bamboo grass, such as spontaneous motor activity (Nagasawa and Hatorri, 2001) and anti-tumor activity (Tsunoda et al., 1998). Many papers have also indicated that bamboo-leaf extract has multiple biological effects, such as anti-free radical, anti-oxidation, anti-aging, and the prevention of cardiovascular diseases (Zhang and Ding, 1996, 1997; Tang and Ding, 2000).

Flavonoids, which are present in the food we eat, have generated particular interest with regard to human health effects, including, for example, antioxidant activities (Pannala et al., 2001; Heim et al., 2002; Bahorun et al., 2004), protection from cardiovascular diseases (Sesso et al., 2003; Wilmsen et al., 2005), and cancer prevention (Kosmider and Osiecka, 2004).

One of the main functional components in bamboo-leaf extracts (BLE) was reported to be flavonoids. There is a close connection between the biological effects of BLE and the activities of flavonoids. The flavonoids in BLE, which are flavone C-glucosides, include orientin, isoorientin, vitexin, and isovitexin, which are considered to be a group of representative flavonoids (Zhang et al., 2005). The extracts from *Phyllostachys nigra* leaves were shown to exhibits antioxidant activities in various different model systems that include DNA, lipoprotein, and complex lipid systems *in vitro* (Hu et al., 2000). However, no studies on the anti-oxidant activities of BLE have been reported yet *in vivo*, especially their roles in the metabolism of lipids in the liver and kidneys.

Free radical chain reactions are one of the major

characteristics of anti-oxidant activity. Free radicals that can cause oxidative damage are byproducts of the normal reactions within the human body. These reactions contribute to the generation of energy, the degradation of lipids, the catecholamine response under stress, and inflammatory processes (Ikeda and Long, 1990). If the balance between the production and eradication of free radicals that can cause oxidative damage is maintained, the harmful effects of free radicals would be minimized in the body. However, if the free radicals are not eradicated efficiently, oxidative stress can occur. Oxidative stress, caused by reactive oxygen or free radicals, has been shown to be associated with the progression of many diseases, including cancer, heart disease, depression, and several others (Kovacic and Jacintho, 2001; McCord, 2000; Parola and Robino, 2001). In order to protect tissues and organs from oxidative damage, the body possesses enzymatic systems. The main enzymes include SOD, GSH-Px, and CAT, which are our frontline defenders against oxidative damage.

First, we investigated the anti-oxidative properties of BLE towards rats *in vivo*. Rats were fed with cholesterol-rich foods containing different amounts of BLE and vitamin E (VE). The enzyme activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), as well as the values of thiobarbituric acid reactive substances (TBARS) in the liver and kidneys, were determined. The radical scavenging activity of DPPH, the total antioxidant capacity and reduction ability, were investigated *in vitro* for use in the subsequent discussion of the anti-oxidative mechanisms associated with BLE.

MATERIALS AND METHODS

Preparation of bamboo leaves extracts

Fresh bamboo leaves from *Dendrocalamopsis oldhami*, a bamboo species that is widely planted in the southern part of China, were collected during the spring and summer seasons in Nanping City, Fujian Province, China. The leaf samples of this species were sent to Xishuangbanna Tropical Botanical Garden of the Chinese Academy of Science in Yunnan Province for final identification. The fresh bamboo leaves were air-dried, cut into small pieces ranging in size from 0.5 to 1.0 cm, and then used to prepare BLE. The coarse pieces of bamboo leaves (200 g) were soaked in 2000 ml of an aqueous solution of ethanol (70% ethanol/30% water, v/v) for 2 h at room temperature (24 ± 2°C) and refluxed for about 3 h in a round flask. The solvent containing BLE was cooled to room temperature and poured out of the round flask, leaving the residue of the bamboo leaves behind. The solvent containing BLE solvent was centrifuged at 4000 *g* for 15 min at room temperature, and, then, the supernatant was concentrated with rotary evaporators equipped with a water bath at 45 to 50°C. Finally, the BLE was obtained by lyophilized preparation.

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Abbreviations: **AOAC,** Association of Analytical Communities; **BLE,** bamboo-leaf extracts; **BSA,** bovine serum albumin; **CAT,** catalase; **DAD,** diode array detector; **DPPH,** 1,1-diphenyl-2-picrylhydrazyl; **GSH-Px,** glutathione peroxidase; **HLBE,** high-dosage bamboo-leaf extract; **HPLC,** high pressure liquid chromatography; **IC_{50},** half maximal inhibitory concentration; **LDBE,** low-dosage bamboo-leaf extract; **MDA,** malondialdehyde; **NADPH,** nicotinamide adenine dinucleotide phosphate; **QE/g BLE,** quercetin equivalent in per gram BLE; **SOD,** superoxide dismutase; TBA, thiobarbituric acid; **TBARS,** thiobarbituric acid reactive substances; **TF,** total flavonoid; **VCE/g BLE,** vitamin C equivalent in per gram BLE; **VE,** vitamin E.

Table 1. Gradient elution procedure of HPLC.

Time (min)	Proportion of solvent (A) (%)	Proportion of solvent (B) (%)
0	20	80
5	25	75
12	25	75
25	47	53
27	100	0
35	100	0
36	20	80
45	20	80

Proximate compositions analysis of the bamboo-leaf extract

Official Methods of the Association of Analytical Communities (AOAC, 2000) were used to analyze the chemical composition of the freeze-dried BLE preparation, including moisture (Vacuum Oven Method 925.09), total soluble sugar (Dische, 1962), protein (Method 955.04 using 6.25 as conversion factor), lipids (Method 945.39), and ash (Method 924.05).

Measuring the flavonoid contents of bamboo-leaf extract

The total flavonoid content in the BLE was determined as follows: an accurately-weighed, 0.2 g portion of the freeze-dried BLE preparation was dissolved in 100 ml of an aqueous solution of methanol (70% methanol/30% water, v/v), and the solution containing the dissolved extract was filtered through a 0.45-µm filter unit. Flavonoids were analyzed quantitatively by high pressure liquid chromatography (HPLC). The Waters HPLC system used included a 2695 pump, a diode array detector (DAD) (Waters 996), and a Dikma Diamonsil C_{18} column (4.6 × 250 mm) (Dima Co., Ltd., Orlando, FL). The mobile phases for the HPLC consisted of solvent (A), which was 0.1% (v/v) acetic acid in a mixture of acetonitrile and methanol (130/95 volume ratio), and solvent (B), which was 0.1% (v/v) acetic acid in filtered MilliQ water. The solvent gradient procedure is illustrated in Table 1. The flow rate was 0.8 ml/min. The column temperature was 40°C, and the absorption was measured at 340 nm. Samples consisting of 20 µl were injected sequentially. Quantitative data for total flavonoids were obtained by comparison to orientin (CAS 28608-75-5, obtained from the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China). Total flavonoids were estimated by summing individual flavonoids.

Table 2. Composition of experimental diets (%, w/w).

Composition	Content
Casein	20
Methionine	0.3
Corn starch	30
Starch sugar	37.2
Lard	5
Cellulose	3
Mineral mixture	3.5
Vitamin mixture [a]	1
Total	100

[a] There was no VE in the vitamin mixture.

Measuring the anti-oxidant activities of the bamboo-leaf extract *in vivo*

Grouping of animals and preparation of diets

The experimental animals, three-week-old, male Wistar rats (80 to 100 g), were purchased from the Laboratory Animal Center of the Academy of Military Medical Sciences of China, Beijing, China. The rats were housed individually in stainless wire netting cages in an air-conditioned room (temperature: 21 to 23°C; relative humidity: 55 to 60%). The rats were randomized into five groups with seven rats in each of the following groups: blank control, negative control, positive control, low-dosage bamboo-leaf extract (LDBE), and high-dosage bamboo-leaf extract (HLBE). Rats in the blank control were fed daily with a basic diet in which cholesterol was not detected and that was not supplemented with any BLE or VE. Rats in the negative control were fed daily with a cholesterol-basic diet that contained 5 g cholesterol per kilogram of basic diet and that was not supplemented with any BLE or VE. Rats in positive control were fed daily with a cholesterol-basic diet and that was supplemented with 2.5 mg of VE per kilogram of body weight. Rats in the LDBE and HLBE groups were fed daily with a cholesterol-basic diet which was not supplemented with any VE and that was supplemented with 100 and 400 mg, respectively, of freeze-dried BLE per kilogram of body weight.

The basic diet of these rats (Licence No. SCKK (Jing) 2006-0003) was purchased from the Institute of Laboratory Animal Science of the Academy of Military Medical Sciences of China, Beijing. The composition of the basic diet is given in Table 2. No cholesterol or VE was detected in the basic diet. Analytical grade VE and cholesterol were obtained from Sigma Chemical (St. Louis, MO, USA). The 0.5% (w/w) cholesterol batches were mixed carefully into the basic diet before the rats were fed. The aqueous solutions of BLE and VE were prepared prior to use. The process of feeding the rats is shown in Table 3. The experimental period lasted for six weeks, and, after that, the rats fasted for 12 h before testing began.

Collection of in vivo samples

The animals were cared for according to the Guiding Principles in the Care and Use of Animals. The experiments were approved by Hebei Center for Disease Control, Hebei Province, China, which is affiliated with the Chinese National Center for Disease Control and Prevention. The rats were anesthetized with diethyl ether, and they were placed on an experimental desk. Blood was collected from a fine catheter inserted into the celiac artery. After the blood sample was collected, 20 ml of normal saline were perfused through the catheter to wash the blood out. Then, the liver and kidneys were harvested. The liver and kidneys were cut into pieces and milled to paste, normal saline solution was added to prepare a 10% solution of tissue homogenate, the tissue homogenate was centrifuged at 2000 *g* for 10 min, and the supernatant was kept. All of the samples of liver and kidney tissue were used to measure the enzymatic activities of SOD and GSH-Px, and to measure the values of TBARS. Then, the 10% tissue homogenate was diluted to a 1% solution with normal saline and homogenized by centrifugation at 2000 *g* for 10 min, and the supernatant was kept. These samples were used to measure the CAT activity.

Measuring protein content

The protein content of each sample was measured using a bovine serum albumin (BSA) protein Assay Kit A045-2 (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China) with BSA as the standard (Smith et al., 1985).

Measuring superoxide dismutase (SOD) activity

SOD activity was determined with SOD Assay Kit A001-1 (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China). Superoxide was generated in xanthing oxidase and hypoxanthine, and the superoxide scavenging effect of serum and tissue was determined according to Oyanagui's method (Oyanagui, 1984). Fifty percent inhibition was defined as one unit of SOD activity. The SOD activity of the liver and kidney tissues was expressed in active units per milligram protein (U/mg protein) of the sample.

Measuring catalase (CAT) activity

Catalase activity was determined with CAT Assay Kit A007-1 (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China) by the colorimetric method. It is based upon the alteration of the optical density of H_2O_2 by the enzymatic decomposition of H_2O_2 resulting from the effect of CAT in the sample (Sumner and Dounce, 1937). The CAT of the hemoglobin was changed to k/g Hb after the 'k' value was determined, taking into account the suitable absorbance for each analysis according to the calculated regression. The CAT activity of liver and kidney tissue was expressed in active units per gram protein (U/g protein) of the sample.

Measuring glutathione peroxidase (GSH-Px) activity

GSH-Px activity was determined with a GSH-Px Assay Kit A005 (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China). The GSH-Px had the ability to decompose hydrogen peroxide (H_2O_2) and other organic hydroperoxides (ROOH). Glutathione must be present to complete the reaction. H_2O_2 was used as the substrate for the glutathione. The extent to which nicotinamide adenine dinucleotide phosphate (NADPH) was consumed in the reaction was used to determine the GSH-Px activity. The GSH-Px activity of liver and kidney tissue was expressed in active units per gram protein (U/g protein) of the sample.

Measuring the values of thiobarbituric acid reactive substances (TBARS)

The value of TBARS, an index of lipid peroxidation, was determined

Table 3. Project of feeding rats.

Parameter	Blank	Negative	Positive	LDBE	HDBE
Basic diet	√[b]	-	-	-	-
Cholesterol-basic diet	-	√[b]	√[b]	√[b]	√[b]
Normal saline	n[c] ml	n[c] ml	-	-	-
VE aqueous solution（0.25 mg/ml）	-	-	n[c] ml	-	-
BLE aqueous solution（10 mg/ml）	-	-	-	n[c] ml	-
BLE aqueous solution（40 mg/ml）	-	-	-	-	n[c] ml

[b] The rats used in the experiment were fed daily with that certain diet. [c] The rats used in the experiment were fed daily with that aqueous solution according to the weight of the rats, that is, n = weight of rat (g) /100 (g). The rats was injected down their throats with a syringe artificially.

with a malondialdehyde (MDA) Assay Kit A003-1 (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China). The value of TBARS was estimated using the thiobarbituric acid (TBA) method (Asakawa and Matsushita, 1980). The samples combined with TBA were heated in an acidic environment. The absorbance of the resulting solution was measured at 532 nm. The value of TBARS was expressed in nmol per milligram protein (nmol/mg protein) of the sample.

Measuring the anti-oxidative activities of bamboo-leaf extract *in vitro*

Measuring the reducing power

Reducing power was determined according to the method of Yen and Chen (1995), and the BLE was diluted with ethanol to final concentrations of 0.4, 0.6, 0.8, 1.0, 1.5, and 2 mg/g. Sample solutions (1 ml) were mixed with phosphate buffer (2.5 ml, 0.2 *M*, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.0 ml, 1%), and the mixtures were incubated at 50°C for 20 min. A 2.5-ml portion of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 *g* for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeC1_3$ (0.5 ml, 0.1% w/v), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Using quercetin as the reference standard, the reducing power of BLE was expressed as mg QE/g BLE from a standard curve made from quercetin.

Measuring 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities

The DPPH (2,2-diphenyl-1-picryl-hydrazylhydrate) radical scavenging activity of the bamboo-leaf extract was determined according to the methods described by Mensor et al. (2001) with some modifications. Sample solutions including freeze-dried powder (BLE) ethanol solution (94.160 mg/g) and the reference standard (quercetin, CAS 117-39-5) ethanol solution (6.640 mg/g) were diluted to final concentrations of 23.540, 11.770, 5.885, 2.943, 1.472, 0.736, 0.368, and 0.184 mg/g in ethanol and concentrations of 2.000, 1.000, 0.500, 0.050, 0.026, and 0.013 mg/g in ethanol, respectively. Quercetin was obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

A volume of 5.8 ml of a 6.5×10^{-4} *M* DPPH ethanol solution was added to 0.2 ml of sample solutions of different concentrations, and the solutions were allowed to react at room temperature. After 30 min, the absorbance values (Ab) were measured at 517 nm and converted into the percentage anti-oxidant activity using the following equation: scavenging capacity % = [1 - (Ab of sample - Ab of blank)/Ab of control] × 100%. Ethanol (5.8 ml) added with 0.2 ml of sample solution was used as a blank, while DPPH solution (5.8 ml, 6.5×10^{-4} *M*) added with 0.2 ml of filtered MilliQ water was used as a control. The 50% inhibiting concentration (IC_{50}) values were calculated by linear regression of plots where the abscissa represented the concentration of BLE or quercetin ethanol solution and the ordinate was the average percentage of scavenging capacity from the three replicates.

Measuring total anti-oxidant capacity

Total anti-oxidant capacity was determined according to the method of Prieto et al. (1999). A volume of 0.1 ml of sample solution containing a certain amount of BLE or VC in ethanol was combined in an Eppendorf tube with 1 ml of reagent solution (0.6 *M* sulfuric acid, 28 m*M* sodium phosphate, and 4 m*M* ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. A typical blank solution contained 1 ml of reagent solution, and 0.1 ml ethanol was incubated under the same conditions as the rest of the samples. The anti-oxidant capacities were expressed as mg VCE/g BLE. VC of analytical grade was obtained from Sigma Chemical (St. Louis, MO, USA).

Statistical analysis

The mean values of each experiment were calculated. The OriginPro system (v8.0988 SR6, OriginLab Corporation, Northampton, MA, USA) was used for statistical analysis. Duncan's multiple range tests were used to estimate significant differences among the mean values at the 5% probability level.

RESULTS

Chemical composition and flavonoid content of the bamboo-leaf extract

BLE prepared from bamboo leaves is a complex mixture. The BLE we tested had total flavonoid (TF) content of 3.7%. Its characteristic spectra of HPLC are shown in Figure 1, which displays the composition of the key flavonoid compounds according to retention times of the

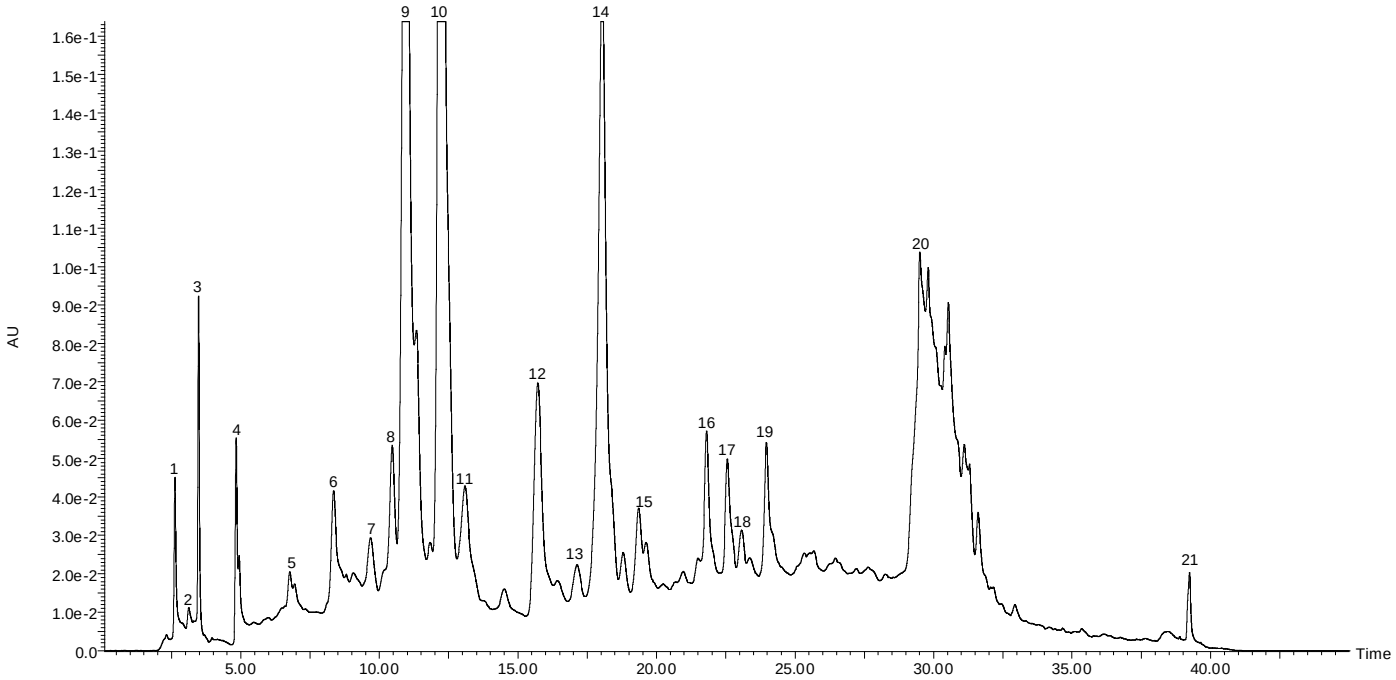

Figure 1. Profiles of bamboo-leaf extracts by high pressure liquid chromatography (HPLC). 1 chlorogenic acid, 2 caffeic acid, 3-5 phenolic acid, 6-8 flavonoid, 9 C-Hexosyl O-pentosyl luteolin, 10 isoorientin, 11 orientin, 12 C-hexosyl O-hexosyl aglycones, 13 vitexin, 14 isovitexin, 15 C-hexosyl luteolin, 16 O-rutinoside apigienin, 17 O-rutinoside tricin, 18 flavonoid, 19 O-hexosyl tricin, 20 luteolin, 21 tricin.

standards and ultraviolet-visible light spectrum data. In addition to flavonoids, the BLE also contained 16% protein, 8.6% fat, 9.6% water, 15% salt, 18% total soluble sugar, and 29% other components.

Anti-oxidant activities of bamboo-leaf extract *in vivo*

Table 4 shows the SOD, CAT, and GSH-Px activities and the values of TBARS in the liver and kidneys. Compared with the controls, the SOD activity of the HDBE group decreased significantly in the liver ($p < 0.05$). The effects in the SOD activities of blank, negative, and positive groups were almost equivalent to the LDBE group in the liver. The HDBE group also showed a significant increase ($p < 0.05$) in the kidneys. In addition, for the LDBE group, the SOD activity in the kidneys was significantly lower than the blank control group and the negative group ($p < 0.05$). The SOD activity of the kidneys for the blank and negative groups increased compared with the positive group, but there was no significant difference ($p > 0.05$). Meanwhile, for the LDBE group, the SOD activity of the kidneys decreased compared with the positive group, but there was no significant difference ($p > 0.05$). For the HDLE group, SOD activity of the kidneys decreased significantly compared with the LDBE control ($p < 0.05$). The SOD activity of the liver and kidneys were lowest in the HDLE group.

The highest CAT activity of the liver was observed in the LDBE group, compared with the blank and positive controls ($p < 0.05$). The CAT activity of the LDBE group was higher than that of the negative and HDBE controls, but there was no significant difference among them ($p > 0.05$).

In the HDBE group, the GSH-Px activity in the liver and kidneys was significantly less that that observed in the positive, LDBE, negative, and blank controls ($p < 0.05$).

The values of TBARS in the HDBE group significantly decreased in the liver compared with the positive and negative controls ($p < 0.05$). Although the values of TBARS in the HDBE group were lower than the blank and LDBE controls, there was no significant difference ($p > 0.05$). The values of TBARS (in LDBE and HDBE groups) of the kidneys decreased significantly compared with the negative, blank, and positive groups ($p < 0.05$). The values of TBARS of the kidneys in the negative group increased, and there were significant differences between this group and the positive, HDBE, and LDBE groups ($p < 0.05$). There was no significant difference compared with the blank group ($p > 0.05$).

Anti-oxidant activities of bamboo-leaf extract *in vitro*

In the evaluation of the anti-oxidant potential for the BLE *in vitro* including the reducing power of BLE, the total

Table 4. The SOD, CAT, and GSH-Px activities and the values of TBARS in the liver and kidneys of cholesterol-fed rats supplemented with vitamin E or bamboo-leaf extract[d, e].

Diet	SOD activities (U/mg protein)	CAT activities (U/g protein)	GSH-Px activities (U/g protein)	TBARS (nmol/mg protein)
Liver				
Blank	200.29 ± 26.59^{A}	45.76 ± 8.33^{B}	660.00 ± 50.46^{A}	6.80 ± 1.19^{BC}
Negative	212.08 ± 22.83^{A}	53.86 ± 16.20^{AB}	658.94 ± 56.15^{A}	11.73 ± 2.62^{A}
Positive	220.27 ± 33.31^{A}	42.45 ± 13.88^{B}	634.19 ± 65.02^{A}	7.32 ± 1.88^{B}
LDBE	205.02 ± 28.40^{A}	58.42 ± 10.98^{A}	651.11 ± 57.29^{A}	6.89 ± 1.22^{BC}
HDBE	167.84 ± 26.95^{B}	47.89 ± 4.43^{AB}	493.38 ± 63.54^{B}	5.18 ± 0.67^{C}
Kidney				
Blank	205.41 ± 13.11^{A}	123.79 ± 25.68^{A}	405.57 ± 69.08^{A}	16.62 ± 2.03^{AB}
Negative	207.60 ± 13.18^{A}	92.71 ± 20.40^{B}	431.06 ± 96.56^{A}	18.27 ± 1.54^{A}
Positive	195.25 ± 12.98^{AB}	100.63 ± 18.27^{B}	399.49 ± 48.71^{A}	14.92 ± 2.02^{B}
LDBE	189.62 ± 19.54^{B}	89.72 ± 16.96^{B}	403.35 ± 73.18^{A}	10.63 ± 2.60^{C}
HDBE	172.53 ± 14.63^{C}	100.29 ± 22.33^{B}	333.90 ± 73.76^{B}	11.18 ± 1.36^{C}

[d] Values represent the mean ± standard deviation of duplicate assays in seven animals in each group. [e] Values in a column with different superscripts were significantly different ($p<0.05$).

anti-oxidant capacity and scavenging activity on DPPH were evident. The reducing power was 74 mg QE/g BLE. Table 5 reports the radical scavenging activities of DPPH and the total anti-oxidant capacity of BLE and quercetin. The average IC_{50} value on DPPH was 2.36 mg/g. Total antioxidant capacity was 84 mg VCE/g BLE.

DISCUSSION

Our study demonstrated that the body weight gain of rats that were fed BLE changed (data not shown). The body weight gain of rats in the HDBE group was prominent, while it was unchanged in the other groups. This result suggests that BLE influenced the body weight gain of the rats.

In our tests, we found that SOD and GSH-Px activities decreased significantly in both the liver tissue and kidney tissue of the rats that received BLE as a part of their diets; even so, no significant decrease in CAT activity was observed in the liver tissue. These results suggest that BLE might have depressed the effects of different anti-oxidant enzymes in the kidneys and might have different effects on these anti-oxidant enzymes in the liver.

The cholesterol-rich diets of rats can cause the increase of lipid peroxidation and expose the animals to oxidative stress (Tasi, 1975). TBARS is a good indicator of lipid peroxidation, and, the liver and kidney tissues, the values of TBARS of the positive control and of two BLE groups decreased significantly (Table 4).

Anti-oxidant enzymes are capable of eliminating reactive oxygen species and lipid peroxidation products, thereby protecting cells and tissues from oxidative damage. Anti-oxidant enzymes include SOD, CAT, and GSH-Px, of which SOD can mutate the superoxide radicals to form molecular oxygen and H_2O_2, and CAT and GSH-Px can decompose H_2O_2 to molecular oxygen and water.

We hypothesize that the main protective role of BLE against oxidative damage *in vivo* might be due to the decrease of free radicals rather than the increase of the activity of anti-oxidant enzymes. It may be that BLE blocks chain reactions of lipid peroxidation in the process of metabolism and thereby plays the role of an anti-oxidative agent. The fewer lipid peroxidation products there are, the less the need for anti-oxidant enzymes becomes. At the same time, we speculate that the changes in enzyme activities are related to the components or metabolites of BLE, which could affect enzymatic activities or enzyme content.

It is known that flavonoids contribute to anti-oxidant activity. Our results showed that the BLE contained 3.7% TF. This TF contained 16 kinds of flavonoid compounds, specifically, five kinds of mono-C-glycosylflavones, two kinds of O,C-Diglycosylflavones, three kinds of O-glycosylflavones, two kinds of flavonoid aglycones, and four kinds of other flavonoids (Figure 1). Flavonoids are a class of compounds that have been demonstrated to be potent antioxidants based on their phenolic hydroxyl groups. Their anti-oxidant activity has been attributed to their electron-donating ability. Studies of the correlation between the structure and activity of flavonoids have shown that the o-dihydroxy structure in the B ring and the 2, 3 double bond in conjugation with the 4-oxo function in the C ring (as in flavones) are essential for effective free radical scavenging activity (Jovanovic et al., 1996; Rice-Evans et al., 1996; Lien et al., 1999; Pietta, 2000).

In order to make sure of the extent to which flavonoids contribute to the anti-oxidant activity of BLE, some *in vitro*

Table 5. DPPH radical scavenging activities and total anti-oxidant capacity in BLE and quercetin.

Parameter	DPPH radical scavenging activities				Total anti-oxidant capacity (mg VCE/g)
	Concentration range (mg/g)	Regression equation [f]	Coefficient of determination (R^2)	IC_{50} [g](mg/g)	
BLE	23.540-0.184	y = 19.864Ln(x) + 32.972	0.9907	2.36	84.0±3.2
Quercetin	2.000-0.013	y = 15.895Ln(x) + 80.331	0.9953	0.15	292.0±5.6

[f] x: the concentration of BLE. y: the average percent of scavenging capacity. [g] Values obtained from regression lines with 95% of confidence level. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum inhibition.

research was conducted. The BLE showed less scavenging activity on DPPH, less total anti-oxidant capacity, and less reducing power than the typical flavonoid compound quercetin (Table 5). However, it was evident that the BLE did show both anti-oxidant activity and pro-oxidant activity *in vitro*. First, the anti-oxidant activity of the BLE might be attributed to its proton-donating ability as evidenced through its DPPH radical scavenging results. Second, the total anti-oxidant capacity of BLE to reduce Mo (VI) to Mo (V) was assayed, indicating the subsequent formation of a green phosphate/Mo (V) complex at acidic pH values. BLE exhibited reducing power as evidenced by the conversion of iron to ferrous ions. Therefore, BLE might have pro-oxidant (electron donor) properties and could react with free radicals, converting them to more stable products and terminating the chain reaction of the free radicals. It was concluded that the BLE has both anti-oxidant activity and pro-oxidant activity; the antioxidant activity was attributed to free radical scavenging, and the pro-oxidant activity, albeit minor, resulted from the reducing power in the presence of transitional metal ions (Hu et al., 2000). Our results agreed with their results in the *in vitro* study. Therefore, the flavonoids might play an important role as anti-oxidant components in BLE.

Table 5 shows that the IC_{50} value (DPPH radical scavenging activities) of the BLE was 2.36 mg/g and that total anti-oxidant capacity was 84 mg VCE/g BLE. According to the content of total TF in BLE was 3.7%, so the IC_{50} value of the TF was 0.087 mg TF/g and that total anti-oxidant capacity was 2270 mg VCE/g TF. The IC_{50} value of the TF (0.087 mg TF/g) was about half of the value of quercetin (0.15 mg /g). The total anti-oxidant capacity of TF (2270 mg VCE/g TF) was nearly 800% greater than the capacity of quercetin (292 mg VCE/g). While the reducing power was 74 mg QE/g BLE, so the reducing power of TF was 2000 mg QE/g TF. Regardless of the DPPH radical scavenging activities, total anti-oxidant capacity, and the reducing power, those powers of the TF were better than those of the typical flavonoid “quercetin”. In section of Chemical composition and flavonoid content of the bamboo-leaf extract, it can been seen that, in addition to flavonoids, the BLE also contained 16% protein, 8.6% fat, 9.6% water, 15% salt, 18% total soluble sugar, and 29% other components. While the flavonoids in BLE might play an important anti-oxidant role, we could also speculate that the other compounds could also play a synergistic role.

Attention has been focused on the oxidative stress caused by a high cholesterol diet, and, after it was found that VE had potential as an anti-oxidant, many dietary supplements have been developed with the same goal in mind. BLE has shown many characteristics of an ideal anti-oxidant material. Bamboo is a giant, woody grass that has a tropical and cosmopolitan distribution and that has become an important commodity. Bamboo leaves have been considered as waste, and they are abundant and cheap. The anti-oxidant property of BLE and its abundance and low cost indicate that it might be useful in slowing the aging process and for treating some chronic diseases. In the absence of comparative investigations and based on these limited experiments, according to the ratio of the weight of the experimental rats to the weight of a person, people might consider consuming 4 mg of BLE per day for each kg body weight (equivalent to 1% of the dose received by the HDBE group) as a dietary supplement as a preventive measure.

Conclusions

Data from the activities of SOD, GSH-Px, and CAT, as well as the values of TBARS in the liver and kidney tissues of tested rats, proved the anti-oxidant effects of the extract of *D. oldhami* bamboo leaves *in vivo*. The BLE had good radical-scavenging activities, total anti-oxidant activities, and reducing power. This present work led us to conclude that BLE from the *D. oldhami* species has excellent anti-oxidant characteristics and an inhibitory effect on lipid peroxidation. Flavonoids, in combination with some of the proteins and sugars from BLE, might be the major contributors to the anti-oxidant activity of BLE.

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