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Evaluation of Antihypertensive and Antihyperlipidemic Effects of Bamboo Shoot Angiotensin Converting Enzyme Inhibitory Peptide in Vivo

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ABSTRACT: Cardiovascular protective functions of bamboo shoot angiotensin converting enzyme (ACE) inhibitory peptide (BSP) from bamboo shoot were evaluated, including antihypertensive effect on spontaneously hypertensive rats (SHRs) and antihyperlipidemic effect on high-fat-diet-induced rats. Thirty-day antihypertensive effects of BSP on SHRs were assayed. Asp-Tyr [10 mg/day kg body weight (BW)] and BSP (50 mg/day kg BW) significantly reduced ACE activity in lung at the same level ($p < 0.05$), while BSP (50 mg/day kg BW) reduced systolic blood pressure (SBP) more effectively for its phenolic compounds, such as ferulic acid and *p*-coumaric acid, which when combined with ACE inhibitor exert a synergistic effect. BSP could significantly reduce SBP, improve oxidant stress status (GSH-Px, SOD, TAC and MDA), and increase NO level in serum and NOS activity in kidney. BSP decreased total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-c) content and MDA level of hyperlipidemic rats, which might contribute to the ACE inhibitory capacity of Asp-Tyr and the fatty acid synthase inhibitory activity of phenolic compounds.

KEYWORDS: *bamboo shoot ACE inhibitory peptide, hypertension, hyperlipidemia, spontaneously hypertensive rats, oxidative stress, fatty acid synthase*

INTRODUCTION

Hypertension is a highly controllable risk associated with cardiovascular diseases such as arteriosclerosis, stroke, and myocardial infarction.[1] Angiotensin converting enzyme (ACE, EC 3.4.15.1) is a dipeptidyl carboxypeptidase involved in different blood pressure regulating mechanisms. Since the first ACE inhibitor peptide was discovered in the venom of the snake *Bothrops jararaca* in the 1970s, a series of ACE inhibitors have been synthesized, such as captopril, lisinopril, and enalapril.[2] However, synthetic ACE inhibitors are associated with a range of side effects, including cough, skin rashes, and reduced renal function.[3] In recent years, more attention has been focused on food-derived antihypertensive peptides by researchers and consumers. Actually, a variety of ACE inhibitory peptides have been identified, such as grass carp fish,[4] digested whey protein,[5] milk protein,[6] and fermented food.[7]

Bamboo shoot, the young stems of bamboo, has been a common vegetable for centuries in China.[8] *Phyllostachys pubescens*, which is widely distributed in China (85%), is the main bamboo producing bamboo shoot. Antioxidant of bamboo leaves (AOB), an extract from *P. pubescens*, has been reported to exhibit multiple biological activities, such as scavenging oxygen radicals and anticancer, antibacterial, and antiviral activitiy, and is especially known for its antioxidant activity.[9,10] Park and John have pointed out that bamboo shoots could reduce the serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and atherogenic index (AI) of young women,[11] and the methanol extract of bamboo shoot exhibited ACE inhibitory activity in vitro,[9] while only in vivo study could confirm the antihypertensive effect of bamboo shoot extracts.

Extract of bamboo shoot has been proved to exhibit ACE inhibitory activity in vitro.[9] Currently, annual production of canned bamboo shoot has been more than 300 000 tons in China. There are many amino acids and phytochemicals in the boiled water produced during canning that have not been utilized and been released randomly, causing environmental pollution. In this paper, the cardiovascular protective functions of bamboo shoot peptide (BSP) were evaluated, including an antihypertensive effect on spontaneously hypertensive rats (SHRs) and an antihyperlipidemic effect on high-fat-diet-induced rats, in order to provide reference for efficient utilization of bamboo shoot.

MATERIALS AND METHODS

Materials. Bamboo (*P. pubescens*) shoot was purchased from Hangzhou U-mate Technology Co., Ltd. (Hangzhou, Zhejiang, China). Aqueous extract of boiled bamboo shoot (AEBS) was collected with volume ratio of 2:1 (water:bamboo shoot, w/w). AEBS was filtrated by a molecular weight cutoff membrane of 5 kDa (Millipore Corp., Billerica, MA) and the permeated fraction was enriched by macroporous resin DA201-C (Jiangyin Chemical Industry Factory, Wuxi, China). After adsorptive equilibration was reached, adsorbate-laden resins were washed by distilled water (1 bed volume/h) until electrical conductivity was equal with that of distilled water.

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Then, the resins were desorbed with 70% ethanol. The absorbate obtained through macroporous resin DA201-C enrichment is bamboo shoot peptide with high ACE inhibitory capability, called BSP for short. BSP, the enriched fraction, was collected, concentrated, lyophilized, and subsequently stored at 4 °C.

Orientin and homoorientin were purchased from Extrasynthese Co. Inc. (Lyon, France). ACE (EC 3.4.14.1) from rabbit lung, HHL (hippuryl-L-histidyl-L-leucine), acetyl-CoA, malonyl-CoA, NADPH, chlorogenic acid, caffeic acid, *p*-coumaric acid, and ferulic acid were all purchased from Sigma-Aldrich Co. (St. Louis, MO). Commercially available kits of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malonaldehyde (MDA), total antioxidant capacity (TAC), nitric oxide (NO), and nitric oxide synthase (NOS) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). Commercially available kits of TC, triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), and LDL-c were purchased from Diasys Diagnostic Systems Co., Ltd. (Shanghai, China). Captopril and lovastatin were provided by Sino American Shanghai Squib Pharmaceutical Co., Ltd., and Zhejiang Hisun Pharmaceuticals Co., Ltd., respectively.

Animals. Spontaneously hypertensive rats (SHRs) (male, $n = 30$), 13−14 week-old and weighting 250 ± 10 g, were provided by Shanghai laboratory animal center (Certificate No. SOXK. 2007-0005, Shanghai, China). Sprague−Dawley rats (male, $n = 60$), with a body weight range of 180 ± 20 g, were provided by Zhejiang University Animal Center (Hangzhou, Zhejiang, China). All procedures were approved by the Care and Use Ethics Committee of Zhejiang University and carried out according to the guidelines established by Experimental Animal Center of Zhejiang University.

Analysis of Amino Acid Composition. Amino acid compositions were analyzed according to Norziah and Ching,[12] using the Waters Associates AccQ Tag method. Identification of amino acids was carried out by comparing the retention time with those of the standards. Materials were dissolved with 6 mol/L of HCl (110 °C, 24 h), and the hydrolysates were analyzed by amino acid analyzer (HP1100, Agilent).

Assay of ACE Inhibitory Activity in Vitro. ACE inhibitory activity in vitro was determined on the basis of the reduced percentage of peak area of hippuric acid, using an adapted HPLC method by Wu and others.[13] The substrate, HHL, was dissolved (5 mmol/L) in 0.1 mol/L sodium borate buffer (pH 8.3) containing 0.3 mol/L NaCl. The assay was performed by mixing 50 μL of substrate solution with 20 μL of inhibitor solution (or borate buffer for control). After incubation at 37 °C for 10 min, 20 μL of ACE solution (100 mU/mL) was added and further incubated at 37 °C for 30 min. The reaction was stopped by the addition of 85 μL of 1 mol/L HCl, and the solution was filtered through 0.45 μm nylon syringe filter membrane before reversed-phase HPLC analysis. HPLC analysis was performed on a C_{18} column (250 mm × 4.6 mm i.d.), particle size 5 μm Varian chromatographic system, and analysis was detected at the wavelength of 228 nm, using captopril as the reference drug.

A blank group was also prepared using the procedure above, but the diluents were replaced by 0.1 mol/L sodium borate buffer (pH 8.3). Mobile phases consisted of solvent A (acetic acid/distilled water, 0.5/99.5, v/v) and solvent B (acetonitrile).

ACE inhibitory rate (%) was calculated as $R = (A - B)/A \times 100\%$, where R is the ACE inhibitory rate of peptide (%), A is the peak area of hippuric acid in the blank, and B is the peak area of hippuric acid in the diluents. The IC_{50} value was defined as the concentration required to reduce the hippuric acid peak by 50% (indicating 50% inhibition of ACE).

Determination of Total Flavonoid and Phenolic Contents. Total flavonoid content was measured using a modified method.[14] Total flavonoid content was determined by a rutin standard curve and expressed as the mean value (g RE/100 g, i.e., gram of rutin equivalent per 100 g of sample) ± SD for three replications.

Total phenolic content of sample was measured with the Folin−Ciocalteu reagent method with modification.[15] Absorbance values were compared with the concentration of the standard (gallic acid). All values were expressed as the mean value (g GAE/100 g, i.e., gram of gallic acid equivalent per 100 g of sample) ± SD for three replications.

HPLC Detection of Phenolic Compounds. Chromatographic separation was performed with a Waters 2996 Photodiode Array Detector (Waters, Milford, MA). HPLC detection of phenolic compounds used a modified method by Zhang.[16] Samples were partitioned with ethyl acetate, lyophilized, dissolved in menthol (about 2.5 mg/mL), and filtered through 0.45 μm membrane filters. Standard solutions of orientin, homoorientin, *p*-coumaric acid, chlorogenic acid, caffeic acid, and ferulic acid were prepared at 1 mg/mL in methanol/water (50/50, v/v). A gradient elution program as follows was used for preparative separation: 15% A (15 min), 15%−40% A (10 min), 40% A (9 min), 40%−15% A (6 min). HPLC analysis was performed at a flow rate of 1 mL/min, with a detection wavelength of 330 nm and temperature of 40 °C.

Thirty-Day Antihypertensive Effect on SHRs. SHRs with SBP higher than 180 mmHg were randomly and equally assigned to five groups for chronic antihypertensive effect ($n = 30$): SHRs control group orally administrated distilled water, positive control group orally administered captopril [10 mg/day kg body weight (BW)], synthetic peptide group orally administered synthetic peptide Asp-Tyr (10 mg/day kg BW), and BSP groups (50, 100 mg/day kg BW). Experimental administration time lasted for 30 days for all groups (shown in Table 2). SHRs were kept at 37 ± 1 °C for 10 min before the measurement of SBP to make the pulsations of the tail artery detectable.

Oxidative Stress Status on 30-Day-Administrated SHRs. After 30 days of feeding, blood was taken from the femoral vein under mild ether anesthesia and centrifuged to separate serum. Activity of SOD and GSH-Px, MDA level in serum and liver, TAC in liver were assayed to reflect oxidative stress status under the guidelines of commercially available kits. NO level in serum and NOS activity in kidney were analyzed.

Determination of ACE Activity in Serum and Lung. ACE activity in serum or lung was measured according to the method of Chshman and Cheung with some modifications.[17] A 100-mg portion of lung tissue was extracted with 500 μL of NP-40 Tris-HCl buffer (pH 7.9) and centrifuged at 13000*g* for 15 min at 4 °C; 100 μL of serum or extract of lung tissue was incubated with 200 μL of 50 mmol/L borate buffer (pH 8.3) containing 300 mmol/L NaCl and 6.5 mmol/L HHL at 37 °C for 30 min. The reaction was terminated with 200 μL of 1 mol/L HCl and then centrifuged at 13000*g* for 15 min at 4 °C. The formed hippuric acid was analyzed by reversed-phase HPLC analysis. HPLC analysis was performed on a C_{18} column (250 mm × 4.6 mm i.d.) of particle size 5 μm. The Varian chromatographic system used detection at a wavelength of 228 nm. One unit (U) of ACE activity was defined as the amount of enzyme that released 1 μmol/L of hippuric acid per min under the conditions.

Antihyperlipidemic Effect on Hyperlipidemic Rats. Male Sprague−Dawley rats ($n = 60$) were equally and randomly divided into six groups after 1-week acclimatization on a basic chow diet (shown in Table 3). The composition of the high-fat diet was as follows: 78.8% basic chow diet, 1% cholesterol, 10% egg yolk powder, 10% lard oil, and 0.2% bile salt. Animals in group 1 were fed with chow diet throughout the experiment period as the normal control. Animals in group 2−6 were fed with high-fat diet for an induction period of 18 days. Subsequently, 42-day oral administration was followed. Animals in group 2−4 were orally administered with high-fat diet and BSP (100, 200, and 350 mg/day kg BW, respectively). Animals in group 5 were orally administered with high-fat diet and lovastatin (10 mg/day kg BW) as the positive control. Animals in group 6 were orally administered with high-fat diet as the model control.

After 42 days of feeding, all animals were fasted for 16 h. Blood was taken from femoral vein under mild ether anesthesia and then centrifuged to separate serum. TC, TG, HDL-c, and LDL-c in serum were determined. Atherogenic index (AI) was calculated as follows

$$\mathrm{AI} = \frac{\mathrm{TC} - \mathrm{HDL\text{-}c}}{\mathrm{HDL\text{-}c}}$$

Oxidative Stress Status on Hyperlipidemic Rats. TAC, SOD, and GSH-Px in serum and MDA level in liver were assayed to reflect

the oxidative stress status under the guideline of commercially available kits.

Determination of Fatty Acid Synthase Activity in Hyperlipidemic Rats. Fatty acid synthase (FAS) activity in liver was determined with the method by Tian[18] with some modifications. Extraction buffer (0.1 mol/L K_3PO_4, 0.07 mol/L $KHCO_3$, 1 mmol/L EDTA and 1 mmol/L DTT, pH 7.8) added at four times the weight compared with the weight of liver sample was mixed with the liver sample of a hyperlipidemic rat. The mixture was then homogenated for 1 min at high speed and centrifugated at 10 000*g* for 30 min at 4 °C, the supernatant of which was collected for FAS activity determination. Twenty microliters of supernatant was added to 2 mL of reaction mixture, which contained 100 mmol/L KH_2PO_4–K_2HPO_4 buffer (pH 7.0), 1 mmol/L EDTA, 1 mmol/L dithiothreitol, 3 μmol/L acetyl-CoA, 10 μmol/L malonyl-CoA, and 35 μmol/L NADPH. Absorption at 340 nm was monitored at a constant temperature of 37 °C. The slope value of the absorption changes in the first minute was used to calculate the initial reaction speed.

Statistical Analysis. All data were expressed as mean ± standard deviation (SD). Differences were analyzed by one-way AVONA using the SPSS package software (SPSS 17.0 for Windows, SPSS Inc., Chicago, IL). Differences were considered significant at $p < 0.05$ and extremely significant at $p < 0.01$.

RESULTS

Amino Acid Composition of BSP. Amino acid composition of BSP possessed relatively high contents of amino acid, especially Tyr in bamboo shoot accorded with the data.[8] Potent ACE-inhibitory peptides are generally short peptides (2–12 amino acids), which usually contain aromatic or hydrophobic residues at their *C*-terminus, such as Pro, Tyr, Val, and Ala residues.[19] Amino acid composition of BSP is shown in Table 1, the higher content of which were Tyr (130.80 ± 1.07 mg/g), Glx (103.05 ± 0.72 mg/g), Asx (60.23 ± 0.74 mg/g), Val (23.70 ± 0.55 mg/g), and Ala (23.56 ± 0.16 mg/g). A key active ACE inhibitory peptide has isolated from BSP in our previous work, with the sequence being Asp-Tyr (isolated from BSP by Sephadex G-15 gel filtration and semipreparative HPLC then characterized by UPLC–ESI-MS).

Table 1. Amino Acid Composition of BSP[a] (mg/g)

amino acid	content
Tyr	130.80 ± 1.07
Glx	103.05 ± 0.72
Asx	60.23 ± 0.74
Val	23.70 ± 0.55
Ala	23.56 ± 0.16
Ser	19.45 ± 0.71
Cys	16.15 ± 0.23
Leu	11.28 ± 0.31
Lys	10.48 ± 0.53
Phe	6.74 ± 0.43
Arg	5.37 ± 0.15
Ile	5.36 ± 0.24
Gly	3.70 ± 0.21
His	3.39 ± 0.07
Pro	2.56 ± 0.08
Thr	1.05 ± 0.07
Met	0.95 ± 0.08
total amino acid contents	427.82 ± 5.75

[a]BSP = bamboo shoot ACE inhibitory peptide. Amino acids composition of BSP: Tyr (130.80 ± 1. 07 mg/g), Glx (103.05 ± 0.72 mg/g), Asx (60.23 ± 0.74 mg/g), Val (23.70 ± 0.55 mg/g), and Ala (23.56 ± 0.16 mg/g). Asx represents both aspartic acid and asparagine. Glx represents both glutamic acid and glutamine.

ACE Inhibitory Activity of BSP in Vitro. ACE catalyzes the degradation of substrate, HHL, so ACE inhibitory activity in vitro was determined on the basis of the reduced percentage of peak area of hippuric acid. Low ACE inhibition IC_{50} means strong ACE inhibitory ability. Park and Jhon have reported that bamboo (*P. pubescens*) shoot extracts possessed ACE inhibitory activity with IC_{50} being 4.7 ± 0.3 mg/mL.[9] ACE inhibitory activity of BSP was higher than that of AEBS (Figure 1)

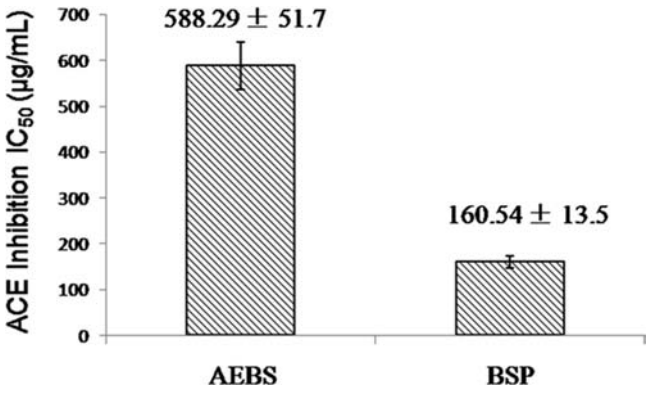

Figure 1. ACE inhibitory activity of BSP in vitro: AEBS = aqueous extracts of boiled bamboo shoots; BSP = bamboo shoot ACE inhibitory peptide which was produced using a molecular weight cutoff membrane of 5 kDa and marcoporous resin DA201-C purification. Bars represent mean ± SD of the IC_{50} of samples. IC_{50} value is defined as the concentration of the sample on its ACE activity of 50% and a lower IC_{50} suggests a stronger antihypertensive activity.

because molecular weight cutoff membranes of 5 kDa and macroporous resin DA201-C enrichment were applied in our study. Molecular weight cutoff membrane and macroporous resin DA201-C enrichment are commonly used in the purification and enrichment of ACE inhibitory peptide.[4,20] Moreover, high salt intake is an important environmental factor to increase blood pressure,[21] while the desalination of macroporous resin DA201-C provided a certain guarantee for the antihypertensive effect of BSP on SHRs. Yokoyama et al. indicated that Asp-Tyr may be the ACE inhibitory center of Asp-Tyr-Gly-Leu-Tyr-Pro originating from dried bonito (*Katsuobushi*).[22] Shiozaki et al. reported an antihypertensive peptide from oyster, Asp-Leu-Thr-Asp-Tyr, that could be digested in vivo to be Asp-Tyr.[23] However, the ACE inhibitory activity in vitro is not always directly related to an antihypertensive effect in vivo,[24] that is to say, an assay to identify whether the key active ACE inhibitory peptide of BSP, Asp-Tyr, could exhibit antihypertensive effect in vivo was required.

HPLC Detection of Phenolic Compounds. The total flavonoid content of BSP was 5.07 ± 0.47 g RE/100 g and the total phenolic content was 10.91 ± 0.98 g GAE/100 g, which indicated that there were high-quality phenolic compounds in BSP. Ferulic acid and *p*-coumaric acid are the major hydroxycinnamic acid derivatives in bamboo cell walls, which participate with lignin to generate polysaccharide–lignin complexes.[25] HPLC chromatogram of BSP was similar to the results of AOB,[16] including homoorientin, orientin, chlorogenic acid, caffeic acid, *p*-coumaric acid, and ferulic acid; however, isovitexin and vitexin were not detected in BSP (Figure 2). The contents of chlorogenic acid, caffeic acid, homoorientin,

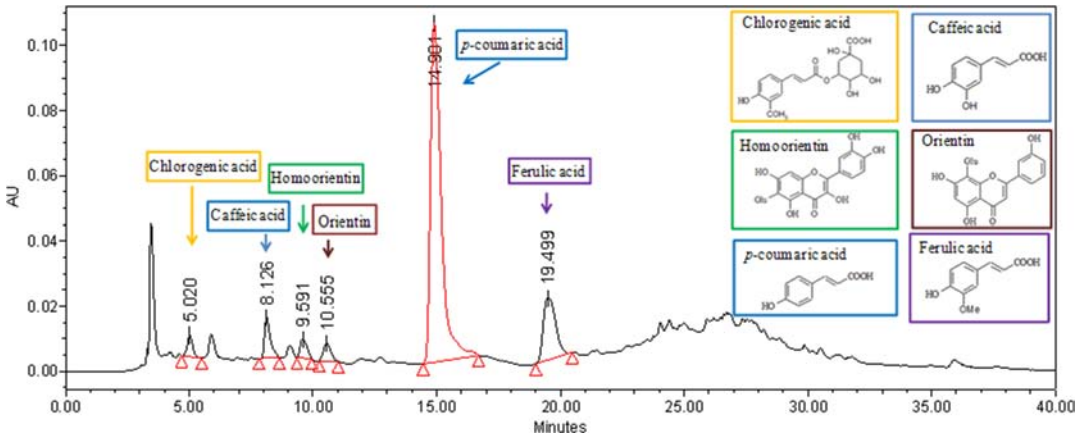

Figure 2. HPLC detection of phenolic compounds: contents of chlorogenic acid, caffeic acid, homoorientin, orientin, *p*-coumaric acid, and ferulic acid in BSP were listed as below (g/100 g, dry weight basis) 0.138 ± 0.003, 0.112 ± 0.002, 0.122 ± 0.005, 0.101 ± 0.02, 1.634 ± 0.031, and 0.250 ± 0.009.

orientin, *p*-coumaric acid, and ferulic acid were listed as below (g/100 g, dry weight basis) 0.138 ± 0.003, 0.112 ± 0.002, 0.122 ± 0.005, 0.101 ± 0.02, 1.634 ± 0.031, and 0.250 ± 0.009.

Thirty-Day Antihypertensive Effect on SHRs. Groups for 30-day antihypertensive effect were as follows: SHR control group, orally administrated distilled water; positive control group, orally administered captopril (10 mg/day kg BW); synthetic peptide group, orally administered synthetic dipeptide Asp-Tyr (10 mg/day kg BW); and BSP groups, two groups of animals orally administered with BSP (50 mg/day kg BW and 100 mg/day kg BW).

Changes in SBP are shown in Figure 3. Compared with control SHRs, SBP decreased after oral administration of Asp-Tyr (10 mg/day kg BW). SBP decreased significantly after oral administration of BSP (50 and 100 mg/day kg BW). SBP declined slowly in the SHRs fed with BSP after 7 d (100 mg/day kg BW), and the difference compared with the control SHRs became more evident during the progress of the experiment. After 21 d of oral administration of BSP (100 mg/day kg BW), SBP tended to be a constant value, which indicated that 30-day intake of BSP will not result in an excessive fall of blood pressure. Nevertheless, captopril (10 mg/day kg BW) was more effective. Some peptides, the compositions of which were Asx, Tyr, Glx, and Val, possess ACE inhibitory activity.[5] Anyway, the key active ACE inhibitory peptide (Asp-Tyr) in BSP reduced the SBP of SHRs in the 30-day antihypertensive test.

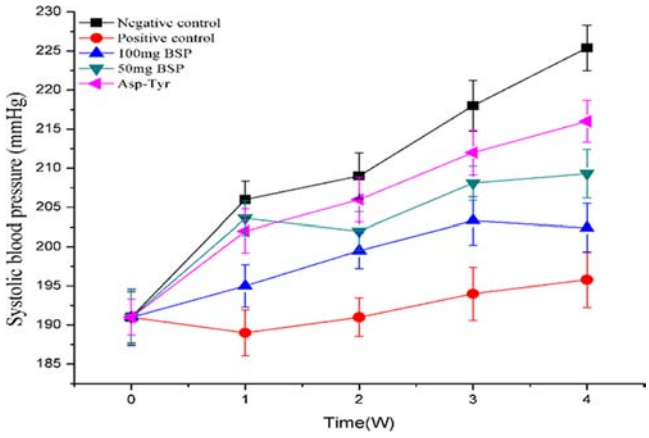

Figure 3. Thirty-day antihypertensive effect of BSP on SHRs: SBP = systolic blood pressure; BSP = bamboo shoot ACE inhibitory peptide; ACE = angiotensin converting enzyme; SHRs = spontaneously hypertensive rats; 100 mg/day kg BW BSP = rats orally administered 100 mg/day kg BW BSP; 50 mg/day kg BW BSP = rats orally administered 50 mg/day kg BW BSP; negative control = rats orally administrated distilled water; Positive control = rats orally administrated 10 mg/day kg BW captopril. Rats were randomly divided into four groups for the 30-day experiment. BSP was diluted with distilled water to prepare BSP solutions of different concentrations. The same amount of distilled water was received by the animals in the negative control group for the substitute of BSP or captopril in the positive control groups. SBP was measured weekly.

Oxidative Stress Status on 30-Day-Administrated SHRs. Oxidative stress status on 30-day-administrated SHRs are shown in Table 2. Compared with SHRs for control, Asp-Tyr (10 mg/day kg BW) administration significantly increased GSH-Px activity ($p < 0.05$). BSP (50 mg/day kg BW) administration significantly increased GSH-Px ($p < 0.01$) and SOD ($p < 0.05$) activity and improved TAC ability ($p < 0.05$) in liver. BSP (100 mg/day kg BW) administration significantly increased GSH-Px and SOD activity ($p < 0.01$) in serum and TAC ability ($p < 0.01$) in liver and reduced MDA level in serum ($p < 0.05$) and liver ($p < 0.01$). Hypertension is closely associated with progressive kidney dysfunction.[26] Overproduction of superoxide and other related reactive oxygen species resulting in oxidative stress reduces the biological effects of nitric oxide.[27] BSP (100 mg/day kg BW) significantly increased the NO level in serum ($p < 0.05$) and NOS activity in kidney ($p < 0.05$), which showed excellent performance on improving oxidative stress status.

Determination of ACE Activity in Serum and Lung. BSP (100 mg/day kg BW) significantly reduced ACE activity in lung ($p < 0.01$) and serum ($p < 0.05$). Asp-Tyr (10 mg/day kg BW) and BSP (50 mg/day kg BW) significantly reduced ACE activity in lung at the same level ($p < 0.05$), while BSP (50 mg/day kg BW) reduced SBP more effectively in 30-day-administrated SHRs. Besides ACE inhibitory peptide, BSP contained a variety of phenolic compounds, such as ferulic acid and *p*-coumaric acid, which could possess antioxidant and antihypertensive function.[28] Antioxidant therapy has been shown to exert beneficial effects in hypertension.[29] The combination of phenolic compounds and ACE inhibitor in BSP exerted a synergistic effect on 30-day-administrated SHRs.

Table 2. 30-Day Antihypertensive Effect of BSP on SHRs[a]

group	NO level serum (μmol/L)	NOS activity kidney (U/mg protein)	ACE activity (mU/mg protein)		GSH-Px activity serum (U/mL)	SOD activity serum (U/mL)	MDA level (nmol/mL)		TAC liver (U/mg protein)
			serum	lung			serum	liver	
SHRs control	30.79 ± 2.54	0.361 ± 0.030	119.43 ± 10.78	164.53 ± 13.07	1417.85 ± 64.94	120.35 ± 9.48	10.98 ± 0.96	2.27 ± 0.12	1.19 ± 0.15
positive control	36.27 ± 3.32[b]	0.449 ± 0.040[b]	104.94 ± 10.02[b]	130.64 ± 10.83[c]	1643.08 ± 87.27[c]	138.97 ± 4.42[b]	9.02 ± 0.65[b]	1.87 ± 0.12[b]	1.42 ± 0.12[b]
Asp-Tyr	31.47 ± 2.76	0.392 ± 0.027	107.66 ± 10.65	146.99 ± 11.38[b]	1587.69 ± 69.85[b]	125.55 ± 5.63	10.11 ± 0.65	2.19 ± 0.13	1.28 ± 0.04
50 mg/day kg BW BSP	33.40 ± 2.93	0.418 ± 0.041	109.68 ± 9.43	138.51 ± 13.03[b]	1598.77 ± 83.87[c]	132.99 ± 7.78[b]	9.89 ± 0.82	2.07 ± 0.17	1.36 ± 0.10[b]
100 mg/day kg BW BSP	34.99 ± 3.28[b]	0.438 ± 0.037[b]	103.24 ± 9.06[b]	134.71 ± 7.96[c]	1739.08 ± 108.78[c]	141.54 ± 6.46[c]	8.43 ± 0.79[b]	1.98 ± 0.14[c]	1.52 ± 0.09[c]

[a]SBP = systolic blood pressure; BSP = bamboo shoot ACE inhibitory peptide; NO = nitric oxide; NOS = nitric oxide synthase; ACE = angiotensin converting enzyme; SHRs = spontaneously hypertensive rats; GSH-Px = glutathione peroxidase; SOD = superoxide dismutase; MDA = malonaldehyde; TAC = total antioxidant capacity; 100 mg/day kg BW BSP = rats orally administered 100 mg/day kg BW BSP; 50 mg/day kg BW BSP = rats orally administered 50 mg/day kg BW BSP; SHRs control = rats orally administrated distilled water; positive control = rats orally administrated 10 mg/day kg BW captopril. [b]$p < 0.05$ compared with the SHRs control group. [c]$p < 0.01$ compared with the SHRs control group.

Table 3. Group Distribution of Hyperlipidemic Rats[a]

group	description	diet
1	normal control	basic chow diet + distilled water
2	100 mg/day kg BW BSP	high-fat diet +100 mg/day kg BW BSP
3	200 mg/day kg BW BSP	high-fat diet +200 mg/day kg BW BSP
4	350 mg/day kg BW BSP	high-fat diet +350 mg/day kg BW BSP
5	positive control	high-fat diet +100 mg/day kg BW lovastatin
6	model control	high-fat diet + distilled water

[a]BSP = bamboo shoot ACE inhibitory peptide. The same amount of distilled water was received by the animals in groups 1 and 6 as as substitute for BSP or lovastatin in other groups, with each group containing 10 rats. All rats were fed for 42 days by oral administration with the treatment shown in the table after an 18-day induction.

Antihyperlipidemic Effect of BSP on Hyperlipidemic Rats. Effect of BSP on TC, TG, HDL-c, and LDL-c in serum of hyperlipidemic rats was tested, as shown in Table 4. Compared with the model group, lovastatin (10 mg/day kg BW) administration could extremely reduce TG ($p < 0.01$), TC ($p < 0.01$), and LDL-c ($p < 0.01$) and increase HDL-c ($p < 0.01$). BSP (100 mg/day kg BW) significantly reduced TC ($p < 0.05$) in high-fat-diet-induced rats. BSP (200 mg/day kg BW) administration significantly reduced TC ($p < 0.01$), TG ($p < 0.05$), and LDL-c ($p < 0.05$). BSP (350 mg/day kg BW) administration significantly reduced TC ($p < 0.01$), TG ($p < 0.05$), and LDL-c ($p < 0.01$). Taking AI for consideration, the effect of BSP (350 mg/day kg BW) administration was most close to the effect of positive group administration, exhibiting an important role in preventing and treatment of atherosclerosis.

Effect of BSP on Oxidative Stress Status in Hyperlipidemic Rats. Similar to previous results of 30-day antihypertensive effect on SHRs, BSP administration could increase the activity of GSH-Px and SOD, increase TAC in serum, and reduce MDA level in liver of high-fat-diet-induced rats (Table 4). Compared with the model control group, BSP (100 mg/day kg BW) could significantly increase the activity of GSH-Px ($p < 0.01$) and SOD ($p < 0.05$) and reduce the MDA level in liver ($p < 0.05$); BSP (200 mg/day kg BW and 350 mg/day kg BW) could significantly increase GSH-Px and SOD activity ($p < 0.01$) and increase TAC ($p < 0.01$) in serum and reduce MDA level in liver ($p < 0.01$).

Effects of BSP on Fatty Acid Synthase in Hyperlipidemic Rats. Compared with the hyperlipidemic rats in the model group, FAS activity of lovastatin-treated rats decreased slightly, but the difference was not significant. However, FAS activity decreased significantly in the BSP groups (200 mg/day kg BW, $p < 0.05$; 350 mg/day kg BW, $p < 0.01$) (Figure 4).

DISCUSSION

Hypertension is a leading cause of cardiovascular (CV) disease, which is the world's largest killer each year.[30] Angiotensin converting enzyme (ACE, EC 3.4.15.1) is a dipeptidyl carboxypeptidase involved in different blood pressure regulating mechanisms. It converts the inactive decapeptide angiothesin I (Ang I) into the potent Ang II in renin−angiotensin−aldosterone system (RAAS), which has been considered as a key point in therapy of hypertension.[31] RAAS plays an important role in development of hypertension's contribution to atherosclerosis.[32] Besides RAAS, many other systems, such as antonomic nervous system, endothelin system,

Table 4. Effect of BSP on Blood Lipid of Hyperlipidemic Rats[a]

group	TC (mmol/L)	TG (mmol/L)	HDL-c (mmol/L)	LDL-c (mmol/L)	AI	GSH-Px activity serum (U/mL)	SOD activity serum (U/mL)	TAC serum (U/mg protein)	MDA level liver (nmol/mg protein)
normal control	1.50 ± 0.11	1.23 ± 0.07	0.89 ± 0.05	0.32 ± 0.03	0.97 ± 0.07	1678.89 ± 74.37	164.90 ± 3.02	12.27 ± 0.59	1.61 ± 0.11
100 mg/day kg BW BSP	2.32 ± 0.19^b	1.61 ± 0.13	0.68 ± 0.06	1.10 ± 0.09	2.27 ± 0.19^b	1358.71 ± 130.14^c	151.98 ± 6.51^b	9.68 ± 0.52	2.28 ± 0.11^b
200 mg/day kg BW BSP	2.15 ± 0.16^c	1.54 ± 0.13^b	0.71 ± 0.07	0.98 ± 0.03^b	1.97 ± 0.19^c	1468.77 ± 94.79^c	161.22 ± 4.60^c	11.85 ± 1.06^c	2.19 ± 0.15^c
350 mg/day kg BW BSP	1.98 ± 0.17^c	1.46 ± 0.10^c	0.72 ± 0.06	0.92 ± 0.09^c	1.38 ± 0.13^c	1535.48 ± 95.16^c	168.13 ± 6.52^c	12.29 ± 0.99^c	1.93 ± 0.13^c
positive control	1.77 ± 0.15^c	1.32 ± 0.12^c	0.81 ± 0.07^c	0.76 ± 0.05^c	1.23 ± 0.11^c	1444.09 ± 68.02^b	152.83 ± 8.10^b	10.98 ± 0.84^b	1.90 ± 0.13^c
model control	2.70 ± 0.18	1.70 ± 0.10	0.65 ± 0.05	1.12 ± 0.07	2.79 ± 0.26	1251.29 ± 74.37	142.74 ± 5.14	9.18 ± 0.88	2.68 ± 0.14

[a]BSP = bamboo shoot ACE inhibitory peptide; GSH-Px = glutathione peroxidase; SOD = superoxide dismutase; MDA = malonaldehyde; TAC = total antioxidant capacity; 100 mg/day kg BW BSP = rats orally administered 100 mg/day kg BW BS; 200 mg/day kg BW BSP = rats orally administered 200 mg/day kg BW BSP; 350 mg/day kg BW BSP = rats orally administered 350 mg/day kg BW BSP; normal control = rats orally administrated distilled water; positive control = rats orally administrated 10 mg/day kg BW lovastatin. $^b p < 0.05$ compared with model control group. $^c p < 0.01$ compared with model control group.

Figure 4. Effect of BSP on FAS activity of hyperlipidemic rat liver: Compared to the model control, FAS activity decreased significantly ($p < 0.05$) in the middle-dose group, and compared with the model group, FAS activity decreased significantly ($p < 0.01$) in the high-dose group.

and nitric oxide system, also contributed to blood pressure control.[33] Ko et al. have reported that NO production via the activation of eNOS is involved in the peptide-induced antihypertensive effect as a vasorelaxation agent.[34] Natural dietary phenolic compounds, such as *p*-coumaric acid, which is the main phenolic compound in BSP and major hydroxycinnamic acid derivatives in bamboo cell walls, could cause endothelium-dependent vasorelaxation in thoracic aorta,[35] which may give some explanation as to why BSP decreased SBP more effectively than the synthetic peptide Asp-Tyr. NO level increased in SHR upon BSP administration, combined with ACE inhibition, to exert a synergistic effect on antihypertension.

Pathogenesis of hypertension is usually associated with oxidative stress, a state of excessive reactive oxidative species activity.[29] In our works, SBP was proved to decline combined with the improvement of oxidative status. ACE inhibitor could reverse high blood pressure by inhibition of lipid peroxidation, up regulation of antioxidant status in hypertension,[36] and reduction of plasma cholesterol in animal models,[37] which have been partially confirmed in our study where Asp-Tyr could improve the oxidative status in SHRs and inhibit lipid peroxidation in hyperlipidemic rats.

Actually, hypertension, hyperlipidemia, and oxidative stress interact with each other in early atherosclerosis.[38,39] Hyperlipidemia is a component of metabolic syndrome associated with lipid abnormalities. The progression of hyperlipidemia is related to lipid peroxidation, increasing the level of free radicals and aggravating hypertension condition and oxidative status. Antioxidants are potential antiatherosclerosis agents, preventing LDL-c from undergoing oxidative modification, which was considered to be a key contributor to atherosclerosis in early lesions,[40] and decreasing oxidative stress.[41] BSP, as a potent natural resource that contained various phenolic compounds, exhibited excellent antioxidant capacity to improve oxidant oxidative status (GSH-Px, SOD, and TAC) both in SHRs and high-fat-diet-induced rats. As an important component of the pathogenesis of atherosclerosis, oxidative stress is always associated with hyperlipidemia and hypertension. It was also reported that ACE expression is enhanced in the coronary artery plaques that contribute to atherosclerosis.[42] The antioxidant capacity of BSP is benefit to exhibit its antihyperlipidemic capacity (TC, TG, and LDL-c) on high-fat-diet-induced fats and antihypertension capacity in SHRs.

Both the antihyperlipidemic and antihypertensive capacity of BSP could play a potent role in preventing atherosclerosis.

Fatty acid synthase (FAS, EC 2.3.1.85) is a lipogenic enzyme involved in energy metabolism of synthesis of long-chain fatty acids and related to various diseases, including obesity, cardiovascular disease, and cancer.[43] Compared with the model control, BSP (200 or 350 mg/day kg BW) could significantly decrease FAS activity of high-fat-diet-induced rats, which may be the role of phenolic compounds contained in BSP. Phenolic components, such as epigallocatechin gallate (EGCG) and epicatechin gallate (ECG), were reported to inhibit FAS in vitro.[18,44] The metabolism of flavone *C*-glucosides and *p*-coumaric acid from AOB was evaluated in rats, in which *p*-coumaric acid could be detected in plasma directly and *C*-glucosides (such as orientin, homoorientin) may exert themselves in the gastrointestinal tract.[45] Several epidemiological studies have suggested a strong link between dietary phytochemical intake and reduced risk for cardiovascular disease.[46,47] Dietary phenolic compounds have been inversely correlated with plasma TC and LDL-c, and the antihyperlipidemia effect of phenolic compounds has been demonstrated in rat[48] and human.[49] Further investigations of the molecular mechanisms of BSP on FAS expression should be performed.

In summary, BSP exhibited excellent antihypertensive and antihyperlipidemic effect in vivo, with the improvement of oxidative status. As a potential ACE inhibitor, Asp-Tyr in BSP could reduce the SBP of SHRs in a 30-day antihypertensive test. Nitric oxide and antioxidant systems were confirmed to be involved in this process. Compared with the synthetic peptide Asp-Tyr, BSP showed stronger antihypertension, which may be a synergistic effect of phenolic compounds and ACE inhibitor. Furthermore, BSP could also reduce TC, TG, and LDL-c in serum and MDA in the liver of high-fat-diet-induced fats, which might own ACE inhibitory activity and antioxidant activity in vivo.

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Notes

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ABBREVIATIONS USED

ACE, angiotensin converting enzyme; AI, atherogenic index; Ang, angiothesin; AOB, antioxidant of bamboo leaves; BSP, bamboo shoot ACE inhibitory peptide; FAS, fatty acid synthase; GSH-Px, glutathione peroxidase; HDL-c, high-density lipoprotein cholesterol; HHL, hippuryl-L-histidyl-L-leucine; HPLC, high-performance liquid chromatography; LDL-c, low-density lipoprotein cholesterol; MDA, malonaldehyde; NO, nitric oxide; NOS, nitric oxide synthase; RAAS, renin–angiotensin–aldosterone system; SBP, systolic blood pressure; SD, standard deviation; SHRs, spontaneously hypertensive rats; SOD, superoxide dismutase; TAC, total antioxidant capacity; TC, total cholesterol; TG, triglyceride.

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