

Full Length Research Paper

Safety evaluation of polyphenol-rich extract from bamboo shavings

Jinyan Gong, Xiaoqin Wu, Baiyi Lu and Ying Zhang*

Department of Food Science and Nutrition, College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310029, Zhejiang Province, PR China.

Accepted 10 September, 2009

In this paper, a number of acute and subchronic toxicological tests were documented to evaluate the safety of a polyphenol-rich ethanol extract of bamboo shavings (EEBS). (i) Acute toxicity test: The oral maximum tolerated dose (MTD) of EEBS was above 20 g/kg body weight for mice, thus the amount can be seen as practically non-toxic. (ii) Mutagenicity test: No mutagenicity was detected according to the negative experimental results of Ames test, micronucleus test on bone marrow cell in mice and abnormality test on mice sperms. (iii) 30 days feeding study: There were no treatment-related toxic effects when EEBS were at levels of 2.50, 5.00, or 10.00 g/kg/day and no adverse effects of EEBS had been observed in these studies. The no-observed-adverse-effect level (NOAEL) of the extract was 10.00 g/kg/day during the 30 days. In addition, no significant distinctions were observed in hematology values, clinical chemistry values and organ/body weight ratio ($P > 0.05$). In conclusion, EEBS is safe and the results support the use of EEBS for various foods.

Key words: Ethanol extract of bamboo shavings (EEBS), safety evaluation, polyphenols, toxicology.

INTRODUCTION

Bamboo shavings (*Caulis Bamfusae* in Taeniam) are retrieved within the second outer layer from the bark of *Bambusa tuldooides* Munro, *Sinocalamus beecheyana* var. *pubescens* P.F. Li or *Phyllostachys nigra* (Lodd.) Munrom var. *henonis* (Mitf.) Stapf ex Rendle, which all belong to the Bambusoideae of Gramineae family. In the long Chinese history, bamboo shavings have been traditionally used as a clinical medicine for alleviating and curing stomachache, diarrhea, vomiting, chest diaphragm inflammation, restlessness and excessive thirst. And its efficacy has been documented in materia medica works from ancient dynasties and recorded in ancient Chinese medicinal books, such as 'Ben Cao Jing Shu', 'Ben Jing Feng Yuan', 'Yao Pin Hua Yi' and 'Bie Lu'.

Abundant bio-active components have been found in bamboo shavings, such as triterpenoids, saponins and sterols. In our preliminary works, it has been clear that

triterpenoids, including friedelin, lupenone, lupenol, α -amyrin and oleanene, were the major active components within the carbon dioxide supercritical fluid extraction technique extract of bamboo shavings (EBS) (Yao et al., 2004; Zhang et al., 2004). The safety of EBS, a triterpenoid-rich extract from bamboo shavings, has been evaluated, too (Zhang et al., 2004). Our previous studies have demonstrated that EBS has excellent anti-fatigue, antihyperlipidemic and antihypertensive activities (Zhang et al., 2006; Jiao et al., 2007a).

In previous experiments, chlorogenic acid, p-coumaric acid and triclin from the leaves of *Phyllostachys nigra* var. *henonis* have been detected and separated (Hu et al., 2000; Jiao et al., 2007b; Zhang Yu et al., 2007). Here, these polar components, such as p-coumaric acid, chlorogenic acid and triclin, were also detected in EEBS, an ethanol extract from the shavings of *Phyllostachys nigra* var. *henonis*. To explore the potential of EEBS for functional food, recently, a series of toxicological tests have been conducted to examine its acute and subchronic toxicity. These studies were performed by independent professionals. All study protocols abide by Good

*Corresponding author. E-mail: yzhang@zju.edu.cn. Tel: Fax: +86 571 8604 9803.

Laboratory Practice (GLP) standards.

MATERIALS AND METHODS

Preparation of EEBS

EEBS was extracted from bamboo shavings of *Phyllostachys nigra* var. *henonis*, which has been identified by Research Institute of Subtropical Forestry, affiliated to the Chinese Academy of Forestry (Hangzhou, China). Fresh bamboo shavings were water-washed and air dried. Coarse powder of bamboo shavings was obtained from comminution and filtration (10 - 20 mesh), then extracted using the carbon dioxide supercritical fluid extraction technique (Zhang et al., 2004). The residual powder was extracted by regurgitant 30% (v/v) ethanol solution for 1.5 h, followed by vacuum concentration and centrifugation separation and dried into the final product by spray dryer.

Characterization of EEBS

The gross amount of flavonoids (TF) and phenolic acids (PA) were measured by photocolometric method (Zhang et al., 2002) with rutin (Sigma-Aldrich, St. Louis, MO, USA) and para-hydroxybenzoic acid (Sigma-Aldrich, Steinheim, Germany) as standards. Analytical HPLC was performed on a Waters 2695 HPLC chromatograph (Waters, Milford, MA, USA) with a Luna C₁₈ column (5 μ m, 250 \times 4.6 mm I.D.) which was purchased from Phenomenex (Torrance, CA, USA). Solvent A (acetonitrile) and solvent B (1% (v/v) acetic acid) were selected as the mobile phases. To analyze column chromatographic fractions, a gradient elution program was used, that is, 15% A (15 min), 15-40% A (10 min), 40% A (9 min), 40-15% A (6 min). The flow rate was 1.0 ml/min, the injection volume was 10 μ l, and the column temperature was maintained at 40°C. The signal was observed at 330 nm using the diode array detector (DAD).

Oral acute toxicity study in mice

20 Kunming male and female mice were obtained from Shanghai Experimental Animal Feed Center, with each mouse's body weight ranging from 19 to 22 g. The mice were housed in cages in a temperature-constant animal room (20-25°C) with a relative humidity of 40 - 70% for 16 h and were fastened overnight but given water *ad libitum* prior to dosage.

The mice were divided into two groups, either group having ten males and ten females. And body weight difference among the same sex does not exceed 3 g. EEBS was dissolved in purified water and controlled by oral gavage at 10 mg/g a dose two times in 24 h. Clinical symptoms, body weight, toxic symptoms and mortality were monitored for two weeks after gavage. Eventually, the maximum tolerated dose and acute toxic classification were confirmed.

Mutation test

Ames test

EEBS was examined for its mutagenic potency in four histidine-requiring *S. typhimurium* mutant strains TA₉₇, TA₉₈, TA₁₀₀ and TA₁₀₂, which were provided by Biochemical Department, University of California (Berkeley, USA) using the treat and plate method (Ames et al., 1975; Maron and Ames., 1983). They were biologically identified before experiment. Sample bacteria were exposed to five concentrations of EEBS, 0.05, 0.15, 0.5, 1.5 and 5 mg/plate, with and without S9 mixture (the liver of rats). Three parallel plates were tested in each concentration. Negative and positive controls

were run simultaneously with the test. All the tests were performed twice.

Mouse micronucleus test

The micronucleus test was based on the method of Hayashi et al. (1990). Fifty Kunming strain mice of both sexes with body weight ranging from 25 to 30 g were conducted in a randomized double-blind manner in this test. EEBS was dissolved in distilled water and administered orally, two times, 30 h apart, at doses of 25.00, 12.50 and 6.25 mg/g body weight to five males and five females each at random. The mice were killed 6 h after the final gavage and their sternum marrow cells were collected and analyzed after methanol fixing and Giemsa staining. The frequency of micronuclei peripheral reticulocytes (MNRETs) was counted based on an examination of 1000 polychromatic erythrocytes (PCE) per mouse. The distilled water and cyclophosphamide (CPA, 0.04 mg/g) was given to another two groups of mice as a negative control and a positive control, respectively.

Mouse sperm abnormality test

The proposed methods (Wyrobek and Bruce, 1975) involve 25 adult male mice weighing from 25 to 35 g. The mice previously obtained were used in this test, which performed in a randomized double-blind manner. Distilled water and cyclophosphamide (CPA, 0.04 mg/g) were respectively given to the mice for five days as a negative control and a positive control. EEBS (25.00, 12.50 and 6.25 mg/g, respectively, in the three remaining groups) was dissolved in distilled water and given to seven randomly selected males by oral gavage for five days. Thereafter, all the mice were maintained on basal diet for 29 days. They were killed by cervical dislocation on the 35th day after the first dose. The bilateral epididymides of five mice were excised and placed in physiological saline and then minced with ophthalmic scissors. Smears were prepared on clean slides, fixed with methanol and stained with 2% eosin. The slides were air-dried and coded for subsequent examination. Morphological evaluation of mice sperms was conducted with a microscope of high magnification. Morphological abnormalities of sperm head shape were assessed according to systematic criteria (Zhang et al., 2004; Ministry of Health PR China, 2003). The number of abnormal sperms out of 1000 sperms was recorded and abnormality was calculated.

Subchronic 30-day oral toxicity study on rats

Previously obtained healthy Sprague-Dawley male and female postweaning rats weighing from 60 to 80 g were divided into four groups, each group having 10 males and 10 females. EEBS was added to a basal feed with concentrations at 0, 2.50, 5.00 and 10.00 g/kg each day and it continued for 30 days (Ministry of Health PR China, 2003). Rats were housed in separate cages. Food and water were provided *ad lib*. Routine clinical observations, body weight and food consumption were measured each week throughout the study period and the total rate of food utilization was calculated. General physical condition of each mouse was observed throughout the test period.

After 30 days of feeding, all of the rats were weighed separately. Blood was sampled by piercing the rat ventral tail vein. Then analyses of hematological parameters were conducted by CD3700 Blood analyzer (Cell-DYN Co., American). At the end of the research, rats were killed by decapitation and clinical chemical values were measured by using CL800 automatic biochemical analyzer (Shimadzu Co., Japan). Liver, kidney, spleen and ovaries (testes) of each rat were observed and weighted and organ/body weight ratios

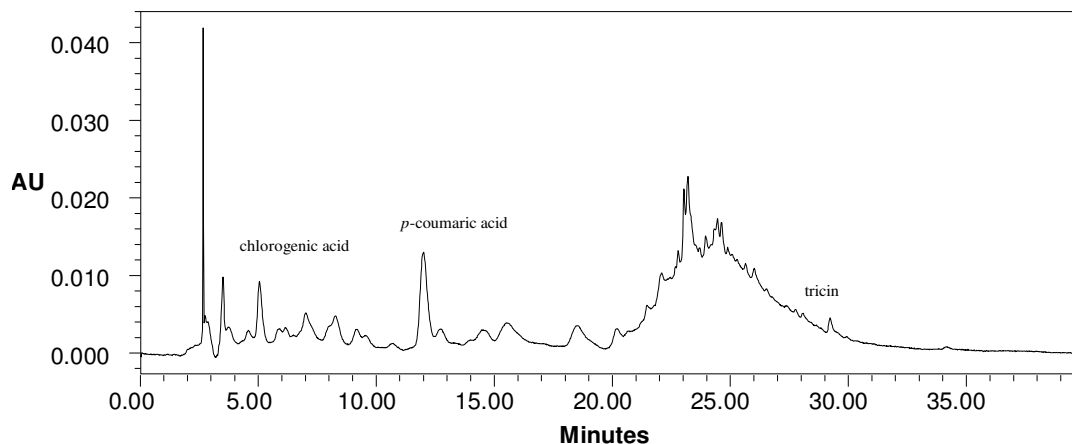


Figure 1. The analytical HPLC chromatograms of EEBS. HPLC column: Luna C18 column (5 μm , 250 \times 4.6 mm I.D.). HPLC column: Luna C18 column (5 μm , 250 \times 4.6 mm I.D.). Mobile phase and gradient programme: A (acetonitrile) and B (1% (v/v) acetic acid); 15% A (15 min), 15–40% A (10 min), 40% A (9 min), 40–15% A (6 min). Flow rate: 1.0 mL/min. Injection volume: 10 μL . Column temperature: 40 $^{\circ}\text{C}$. Wavelength monitoring: 330 nm.

Table 1. Specifications of EEBS.

Parameters	Specification	Methods
Total flavonoids	15.6 %	Photocolorimetric method with lutein standard
Phenolic acids	25.0%	Photocolorimetric method with para-hydroxybenzoic acid standard
Ash	9.1%	Ignition at 550 $^{\circ}\text{C}$
Protein	9.4%	Kjeldahl
Total sugar	29.0%	Anthrone Colorimetry with glucose standard
Total heavy metals (Pb)	<0.001	Atomic absorption spectroscopy
As	<0.0003	Atomic absorption spectroscopy
Moisture	5.0%	Air oven

were calculated. These organs were also examined histopathologically (paraffin section, H-E staining and photomicroscope detection).

Statistical analysis

Parametric ANOVA method was used in all the toxicology tests above. Chi-square test and rank test were used in mouse micronucleus test and sperm abnormality test. Data obtained from other tests were statistically analyzed by *F*-test.

RESULTS

Characterization of EEBS

EEBS is a phenolic fraction of bamboo shavings, the major effective components of which were flavonoids and phenolic acids. The total amounts of flavonoids (TF) and phenolic acids (PA) were determined by using two types of photometric methods. EEBS was identified by reversed HPLC, which was shown in Figure 1. And this EEBS sample contains 15.6% TF and 25.0% PA. In addition,

it contains 5.0% moisture, 9.4% protein, 29.0% sugar and 9.1% ash. The compositions of EEBS sample were given in Table 1.

Oral acute toxicity study in mice

None of the mice died in any of the groups administered with EEBS, regardless of its sex. The general condition of the mice was normal (The data not shown). The maximum tolerated dose of EEBS was more than 20.00 g/kg on mice. Thus, the oral acute toxicity in mice was actually not toxic according to the criteria of acute toxic classifications (Ministry of Health PR China, 2003).

Ames test

Mutagenicity of EEBS was evaluated in a bacterial reverse mutation assay using four *Salmonella typhimurium* strains (TA97, TA98, TA100 and TA102). The reverse mutation test did not show any significant increase in the number of revertant colonies in any strain, whether

Table 2. Ames test results for EEBS in four strains of *Salmonella typhimurium*.

Strain	Dose (mg/plate)	Revertant colonies (mean±SD)			
		-S ₉		+S ₉	
		Test 1	Test 2	Test 1	Test 2
TA ₉₇	0	125 ± 6	117 ± 7	130 ± 10	123 ± 6
	0.05	132 ± 4	123 ± 7	137 ± 5	121 ± 5
	0.15	124 ± 7	128 ± 5	130 ± 5	117 ± 8
	0.50	133 ± 9	130 ± 7	143 ± 7	134 ± 6
	1.50	140 ± 6	116 ± 3	134 ± 6	109 ± 8
	5.00	125 ± 8	116 ± 12	122 ± 6	121 ± 5
	ICR-191 ^a	1626 ± 88	1484 ± 177		
	2-AF ^a			1597 ± 96	1607 ± 145
TA ₉₈	0	38 ± 3	41 ± 3	40 ± 4	40 ± 5
	0.05	38 ± 3	37 ± 5	37 ± 5	38 ± 4
	0.15	39 ± 6	38 ± 5	38 ± 6	39 ± 5
	0.50	36 ± 4	40 ± 3	40 ± 4	37 ± 6
	1.50	40 ± 5	41 ± 4	39 ± 3	40 ± 3
	5.00	37 ± 3	40 ± 3	38 ± 6	41 ± 1
	P-NQ ^a	1097 ± 90	1120 ± 57		
	2-AF ^a			4007 ± 383	4308 ± 365
TA ₁₀₀	0	139 ± 7	138 ± 8	141 ± 5	137 ± 7
	0.05	138 ± 5	136 ± 6	146 ± 6	143 ± 9
	0.15	146 ± 7	137 ± 8	139 ± 10	135 ± 7
	0.50	142 ± 8	141 ± 7	153 ± 13	143 ± 6
	1.50	135 ± 6	140 ± 4	140 ± 7	137 ± 8
	5.00	141 ± 8	135 ± 7	129 ± 6	137 ± 7
	MMS ^a	2483 ± 151	2542 ± 155		
	2-AF ^a			2228 ± 237	2655 ± 341
TA ₁₀₂	0	254 ± 11	257 ± 10	257 ± 12	262 ± 7
	0.05	264 ± 12	261 ± 6	265 ± 13	267 ± 6
	0.15	243 ± 8	258 ± 10	247 ± 13	248 ± 12
	0.50	271 ± 9	263 ± 7	263 ± 22	260 ± 6
	1.50	262 ± 8	245 ± 6	266 ± 11	246 ± 11
	5.00	257 ± 6	251 ± 8	249 ± 14	253 ± 16
	MMS ^a	3856 ± 302	3909 ± 316		
	1,8-DAA ^a			1043 ± 49	1048 ± 83

^a1 µg/plate acridine mutagen (ICR-191), 1 µl/plate 2-aminofluorene (2-AF), 200 µg/plate of p-nitroquinoline (P-NQ), 1 µl/plate methyl methanesulphonate (MMS) and 50 µg/plate 1,8-dihydroxyanthraquinone (1,8-DAA) were used as positive controls. All the tests were performed twice.

with S9 mix or not ($P > 0.05$) (Table 2). Result for EEBS in Ames test was negative according to the data.

Mouse micronucleus test

No significant differences in the density of MN or other clinic data had been found between the EEBS groups and the control group during the test ($P > 0.05$) (Table 3). The result of mouse micronucleus test for EEBS turned out to

be negative.

Mouse sperm abnormality test

The densities of the three groups of doses were 10.4, 10.2 and 10.4% in the sperm abnormalities test (Table 4). The density of the negative group was not too far from the above ($P > 0.05$) but was much lower than that of the positive controls ($P < 0.01$) (Table 4). Data showed that the frequency of sperm abnormality was not significantly

Table 3. Result of micronucleus test for EEBS using mice.

Dose (mg/kg)	No. mice ^a	No. PCE _a	No. MNPCE ^a	PCE/NCE ^a (mean ± SD)	MN ^a (%) (mean ± SD)	P value
Males						
0	5	5000	5	1.10 ± 0.03	1.0 ± 0.71	
2500	5	5000	5	1.09 ± 0.04	1.0 ± 0.45	>0.05
5000	5	5000	5	1.09 ± 0.02	1.0 ± 0.45	>0.05
10000	5	5000	4	1.10 ± 0.04	0.8 ± 0.45	>0.05
CPA(40 mg/kg) ^b	5	5000	134	0.98 ± 0.08	26.8 ± 2.17	<0.01
Females						
0	5	5000	4	1.09 ± 0.03	0.8 ± 0.45	
2500	5	5000	5	1.11 ± 0.05	1.0 ± 0.71	>0.05
5000	5	5000	5	1.09 ± 0.03	1.0 ± 0.71	>0.05
10000	5	5000	5	1.10 ± 0.04	1.0 ± 0.71	>0.05
CPA(40 mg/kg) ^b	5	5000	135	0.99 ± 0.07	27.01.0 ± 2.83	<0.01

^aPCE = polychromatic erythrocytes, MN = frequencies of micronucleus, NCE=normal chromatic erythrocytes.

^bCyclophosphamide (CPA) was treated to mice as the positive control. Distilled water was treated as the negative control.

Table 4. Result of sperm abnormality test for EEBS using mice.

Dose(mg/kg)	No. mice	No. spermatozoa	No. abnormalities	FSA ^a (%)	P value
0	5	5000	50	10.0	
2500	5	5000	52	10.4	>0.05
5000	5	5000	51	10.2	>0.05
10000	5	5000	52	10.4	>0.05
CPA (40 mg/kg) ^b	5	5000	276	55.2	<0.01

^a FSA = frequencies of sperm abnormalities.

^b Cyclophosphamide (CPA) was treated to mice as the positive control. Distilled water was treated as the negative control.

affected by EEBS.

30-day feeding study on rats

General observations

Throughout the 30-day feeding study, there was no death observed in any group. No clinical symptoms were deemed to be related to the feeding of EEBS.

Body weight, food consumption and food availability

No significant difference among the EEBS groups and the control group was observed during the study on body weight (Figures 2 and 3). There was no significant difference among groups on the increase of average body weight, average food consumption and average food availability (Table 5).

Organ weight, organ relative weight

No difference among groups on organ weight and organ/body weight ratio (Table 6) has been observed. Organ

weight and organ/body weight ratio were not significantly affected to EEBS based on the data ($P > 0.05$).

Effects on hematology of rats

No difference among groups on hematology values (Table 7) was observed. Hematology values like WBC, RBC, HGB, LLC, NLC, MLC, ELC and BLC differential counts were not significantly affected by EEBS based on the data ($P > 0.05$).

Effects on clinical chemistry of rats

No difference among groups on clinical chemistry values (Table 8) was observed. Clinical chemistry values like ALT, AST, BUN, creatinine, total cholesterol, triglycerides, glucose, total protein, albumin, globulin and albumin/globulin ratio were not significantly affected by EEBS based on the data ($P > 0.05$).

Histopathological examination on rats

No treatment-related change was observed during

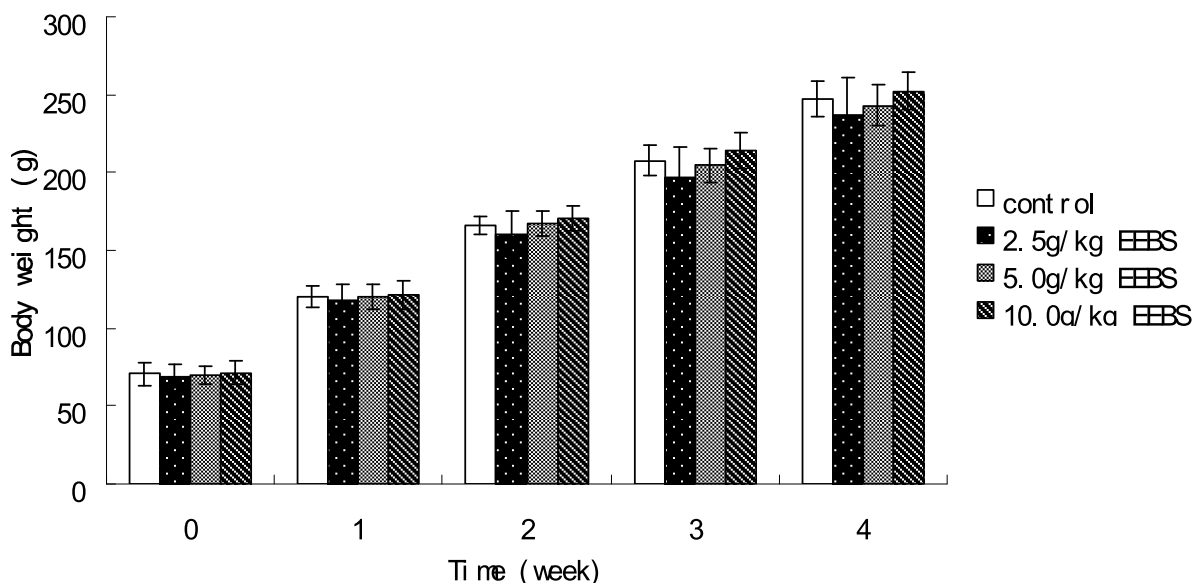


Figure 2. Body weight changes of male rats fed diets containing EEBS for 30 days

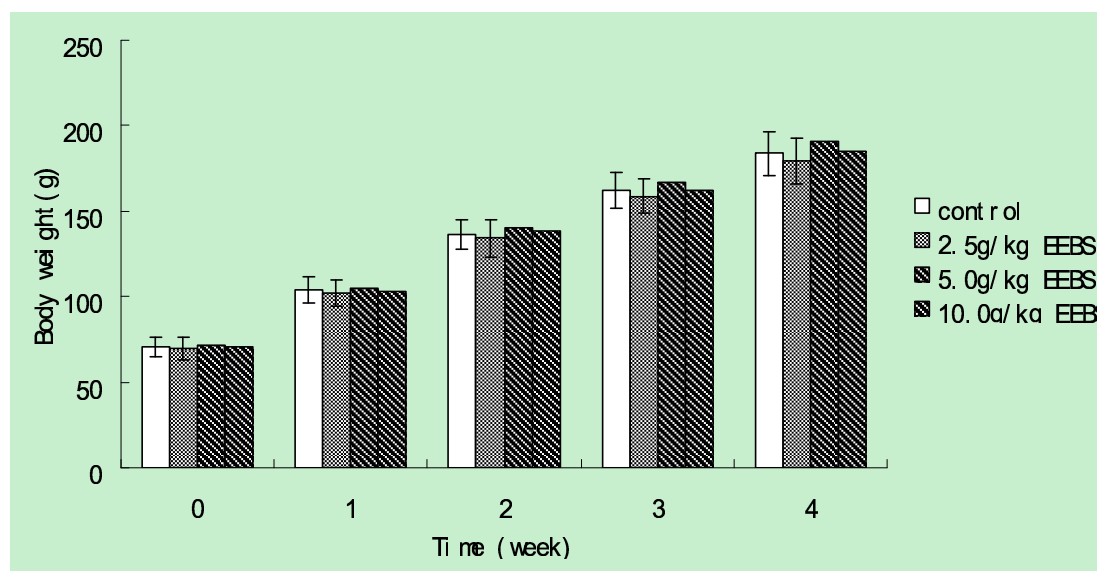


Figure 3. Body weight changes of female rats fed diets containing EEBS for 30 days.

necropsy. Some microscopic changes were observed in some organs from both males and females in the control group and in the high-dose group; however, considering the minor incidence, nothing was notable. No treatment-related change in histopathological examination was observed.

DISCUSSION

Bamboo shavings is clinically used as a traditional Chinese medicine. It is widely accepted that bamboo shavings

contains an abundant amount of biologically active components. Pentacyclic triterpenoid extract from bamboo shavings (EBS) has great potential in many fields for its triterpenoid components which has anti-fatigue functions (Zhang et al., 2006). Furthermore, EBS reduced the total cholesterol (TC) and total triglyceride (TG) on hyperlipidemic rats ($p < 0.05$) and released systolic pressure on spontaneously hypertensive rats (Jiao et al., 2007a). Friedelin, a major triterpenoid compound from EBS, probably has a vasodilator function in thoracic aortas of the rats, which testified the antihypertensive effects of EBS (Jiao et al., 2007a).

Table 5. Body weight increase, food consumption and food availability of rats fed EEBS for 30 days.

Parameter	EEBS in the diet (g/kg)			
	0	2.5	5.0	10.0
Males				
BWI (g)	176.7 ± 6.84	167.6 ± 17.54	173.4 ± 10.70	181.0 ± 7.11
FC (g)	530.2 ± 28.48	498.0 ± 45.43	526.4 ± 21.30	532.8 ± 21.84
FA (%)	33.4 ± 0.88	33.7 ± 1.42	32.9 ± 1.30	34.0 ± 0.63
Females				
BWI (g)	113.4 ± 8.39	109.9 ± 7.90	118.8 ± 8.41	114.5 ± 5.96
FC (g)	513.0 ± 51.20	478.1 ± 29.38	552.4 ± 34.64	506.9 ± 33.33
FA (%)	22.2 ± 0.92	23.0 ± 1.56	21.5 ± 1.59	22.6 ± 1.04

Parameters: body weight increase (BWI), food consumption (FC) and food availability (FA). Ten rats were observed and tested for each group during the experimental period.

Table 6. Organs weight, organs relative weight of rats fed EEBS for 30 days.

Parameter	EEBS in the diet (g/kg)			
	0	2.5	5.0	10.0
Males				
LW (g)	11.71 ± 1.02	11.95 ± 0.76	12.01 ± 1.16	11.64 ± 1.05
KW (g)	2.50 ± 0.11	2.52 ± 0.13	2.53 ± 0.10	2.49 ± 0.10
SW (g)	0.95 ± 0.08	0.92 ± 0.08	0.99 ± 0.07	0.99 ± 0.07
TW (g)	3.42 ± 0.18	3.43 ± 0.20	3.37 ± 0.15	3.42 ± 0.12
LW/BW (%)	4.74 ± 0.40	5.07 ± 0.33	4.94 ± 0.46	4.64 ± 0.59
KW/BW (%)	1.01 ± 0.07	1.07 ± 0.10	1.04 ± 0.05	0.99 ± 0.05
SW/BW (%)	0.38 ± 0.03	0.39 ± 0.04	0.41 ± 0.03	0.39 ± 0.03
TW/BW (%)	1.39 ± 0.12	1.46 ± 0.17	1.39 ± 0.10	1.36 ± 0.09
Females				
LW (g)	7.30 ± 1.04	7.26 ± 0.60	7.63 ± 0.88	7.65 ± 0.67
KW (g)	1.75 ± 0.13	1.62 ± 0.11	1.69 ± 0.11	1.66 ± 0.13
SW (g)	0.67 ± 0.09	0.60 ± 0.07	0.63 ± 0.08	0.60 ± 0.06
OW (g)	0.16 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.01
LW/BW (%)	3.98 ± 0.56	4.06 ± 0.40	4.00 ± 0.44	4.14 ± 0.38
KW/BW (%)	0.95 ± 0.07	0.91 ± 0.08	0.89 ± 0.07	0.90 ± 0.10
SW/BW (%)	0.36 ± 0.05	0.33 ± 0.04	0.33 ± 0.04	0.33 ± 0.03
OW/BW (%)	0.09 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01

Parameters: liver weight (LW), kidney weight (KW), spleen weight (SW), testicle weight (TW), ovary weight (OW), body weight (BW). Ten rats were observed and tested for each group during the experimental period.

Phytophenolic compounds (flavonoids, phenolic acids, etc.) are common dietary components and are present in many beverages and foods, such as red wine, green tea, chocolate, grapes and apples. The average consumption of polyphenols with the diet is 1g/d (Shoji et al., 2004), which have potent biological functions as antioxidant, anti-carcinogen, cardioprotection, photoprotection, etc. (Zern and Fernandez, 2005; Khan and Mukhtar, 2007; Yusuf et al., 2007). Antioxidant of bamboo leaves (AOB) is a bamboo polyphenol preparation which contains flavone C-glycosides and cinnamic acid derivatives, such as

orientin, homoorientin, vitexin, isovitexin, triclin, caffeic acid, chlorogenic acid and *p*-coumaric acid (Zhang, 1995; Zhang et al., 2002). AOB has recently been qualified as a novel natural antioxidant by the Ministry of Health of the People's Republic of China and has been applied to a variety of food products (Lu et al., 2005, 2006). Ethanol extract of bamboo shavings (EEBS) is a new type of bamboo phenolic preparation, which contains chlorogenic acid, *p*-coumaric acid and triclin, with total phenols above 40%. The *p*-coumaric acid, a phenolic acid, occurs in several plant species, consequently, in many foods and beve-

Table 7. Hematological values of rats fed EEBS for 30 days.

Parameter	EEBS in the diet (g/kg)			
	0	2.5	5.0	10.0
Males				
WBC ($\times 10^9/l$)	9.60 \pm 2.90	10.20 \pm 1.47	8.30 \pm 3.12	11.32 \pm 3.13
RBC ($\times 10^{12}/l$)	6.87 \pm 0.81	7.05 \pm 0.61	6.50 \pm 0.63	6.25 \pm 0.74
HGB (g/l)	126 \pm 4.60	130 \pm 7.50	129 \pm 8.1	128 \pm 5.4
LLC (%)	80.0 \pm 2.13	79.5 \pm 1.56	81.4 \pm 2.27	79.4 \pm 1.90
NLC (%)	14.6 \pm 2.20	14.8 \pm 1.63	13.2 \pm 2.14	15.1 \pm 2.28
MLC (%)	3.70 \pm 0.61	4.0 \pm 0.43	3.8 \pm 0.45	3.8 \pm 0.53
ELC (%)	0.8 \pm 0.13	0.8 \pm 0.20	0.8 \pm 0.15	0.8 \pm 0.11
BLC (%)	0.9 \pm 0.16	0.9 \pm 0.17	0.8 \pm 0.27	0.9 \pm 0.19
Females				
WBC ($\times 10^9/l$)	10.10 \pm 3.23	11.31 \pm 3.07	8.67 \pm 2.00	10.70 \pm 3.18
RBC ($\times 10^{12}/l$)	6.47 \pm 0.78	6.56 \pm 0.59	7.00 \pm 0.72	6.13 \pm 0.78
HGB (g/l)	129 \pm 8.7	128 \pm 9.8	129 \pm 6.4	131 \pm 7.4
LLC (%)	80.2 \pm 2.41	81.3 \pm 1.68	79.6 \pm 1.79	80.3 \pm 1.29
NLC (%)	14.1 \pm 2.48	12.7 \pm 1.44	14.7 \pm 1.80	14.1 \pm 1.33
MLC (%)	4.0 \pm 0.41	4.4 \pm 4.38	3.9 \pm 3.90	4.0 \pm 4.03
ELC (%)	0.9 \pm 0.23	0.8 \pm 0.16	1.0 \pm 0.18	0.8 \pm 0.17
BLC (%)	0.8 \pm 0.14	0.8 \pm 0.20	0.8 \pm 0.26	0.8 \pm 0.19

Parameters: white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), lymph leukocyte count (LLC), neutrophil leucocyte count (NLC), mononuclear leukocyte count (MLC), eosinophil leukocyte count (ELC) and basophil leukocyte count (BLC)

Table 8. Clinical chemistry values of rats fed EEBS for 30 days.

Parameter	EEBS in the diet (g/kg)			
	0	2.5	5.0	10.0
Males				
ALB (g/l)	41.0 \pm 3.34	41.3 \pm 2.38	39.0 \pm 2.79	39.8 \pm 1.61
TP (g/l)	71.2 \pm 3.05	68.6 \pm 3.02	72.0 \pm 2.29	69.2 \pm 2.35
CRE (μ M)	40.9 \pm 4.29	42.5 \pm 3.67	37.7 \pm 3.98	39.5 \pm 3.54
AST (IU/l)	133 \pm 9.08	135 \pm 9.94	136 \pm 5.08	128 \pm 5.86
ALT (IU/l)	40.0 \pm 5.61	41.4 \pm 5.36	39.4 \pm 6.62	37.9 \pm 4.38
Glu (mM)	6.37 \pm 0.58	6.66 \pm 0.36	6.59 \pm 0.43	6.42 \pm 0.51
TC (mM)	1.61 \pm 0.16	1.77 \pm 0.21	1.75 \pm 0.16	1.62 \pm 0.26
TG (mM)	1.12 \pm 0.16	1.07 \pm 0.18	1.10 \pm 0.20	1.18 \pm 0.18
BUN (mM)	6.87 \pm 0.35	6.62 \pm 0.76	7.07 \pm 0.49	6.70 \pm 0.73
Females				
ALB (g/l)	40.5 \pm 3.17	38.7 \pm 2.57	41.1 \pm 2.41	40.4 \pm 2.89
TP (g/l)	70.3 \pm 3.70	72.0 \pm 3.17	68.7 \pm 2.45	71.0 \pm 1.55
CRE (μ M)	39.9 \pm 5.50	42.4 \pm 5.88	36.6 \pm 3.15	40.6 \pm 5.31
AST (IU/l)	135 \pm 10.52	141 \pm 9.89	128 \pm 11.16	133 \pm 12.16
ALT (IU/l)	40.0 \pm 6.55	37.4 \pm 3.09	41.1 \pm 6.00	36.4 \pm 3.06
Glu (mM)	6.50 \pm 0.49	6.60 \pm 0.58	6.80 \pm 0.37	6.49 \pm 0.49
TC (mM)	1.68 \pm 0.26	1.67 \pm 0.15	1.70 \pm 0.15	1.72 \pm 0.18
TG (mM)	1.02 \pm 0.23	1.04 \pm 0.21	1.20 \pm 0.17	0.97 \pm 0.16
BUN (mM)	6.85 \pm 0.69	7.04 \pm 0.54	6.30 \pm 0.90	6.53 \pm 0.71

Parameters: albumin (ALB), total protein (TP), creatinine (CRE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose (Glu), total cholesterol (TC) and triglycerides (TG), blood urea nitrogen (BUN).

rages of vegetable origin. Many original contributions reported the great potential of it on antioxidant. It has been demonstrated that *p*-coumaric acid protected against oxidative stress and genotoxicity in cultured mammalian cells, ameliorated the cardiac content of glutathione, superoxide dismutase and catalase activities, decreased accumulation of cardiac content of MDA and protected rat's heart against doxorubicin-induced oxidative stress (Abdel-Wahab et al., 2003; Ferguson et al., 2005). It also (50 mg/kg) significantly decreased the basal level of the oxidative damage assessed as 8-OH-2'-deoxyguanosine levels in DNA and by the comet assay. The data suggest that *p*-coumaric acid acts as an antioxidant in the colonic mucosa in vivo (Guglielmi et al., 2003) and it also has good antiplatelet activity, which suggesting possible applications for primary prevention of vascular disease (Luceri et al., 2007). Tricin is a flavonoid, which has great potential property in the cancer chemopreventive of animals or human cancer cell lines in many original contributions (Jiao et al., 2007b). Tricin interfered potently with the growth of human-derived malignant MDA-MB-468 breast cancer cells, but was much less growth-inhibitory in HBL-100 cells (Hudson et al., 2000). Mechanistic studies found the ability of tricetin to inhibit cell growth and cell cycle-arresting properties in human MDA-MB-468 breast cancer cells *in vitro* (Cai et al., 2004). And high levels of tricetin has been found in the gastrointestinal tract, which can inhibit cyclooxygenase enzymes and attenuate modulation of cyclooxygenase-mediated prostaglandin production, and may contribute to its chemopreventive efficacy on intestinal carcinogenesis and prostatic cancer, respectively (Cai et al., 2005; Al-Fayez et al., 2006).

The main purpose of this study was to establish a safety standard for the use of EEBS which was obtained by using ethanol solution after carbon dioxide supercritical fluid extraction technique extract. Based on the result of Ames test, no reproducible or density-related increase on revertants in prototrophy has been observed in any of the TA strains exposed to EEBS, whether with S-9 mix or not. Therefore EEBS was not considered a mutagenicity-inducing agent. Oral acute toxicity study shows that the maximum tolerated dose of EEBS was above 20 g/kg for both male and female mice. The result indicated a potential for the bamboo extract EEBS in fields such like food supplement. The 30-day oral gavage of EEBS on rats at 2.50, 5.00 and 10.00 g/kg per day did not cause significant adverse toxicological effect that can be attributed to the treatment.

No salient toxicity has also been observed during the 30-day feeding study on rats. Neither has significant difference been observed on organ weight. No dose dependency and hardly any adverse effect have been observed histopathologically. Therefore these were not considered treatment-related effects. The largest dose does not induce noticeable symptoms of toxicity. The no-observed-adverse effect level (NOAEL) of EEBS in the sub-acute toxicity study was equal to 10.00 g/kg of body

weight while the maximum tolerated dose of EEBS in the acute toxicity test was more than 20.00 g/kg of body weight. These results suggest that EEBS was of low toxicity and has a potential in functional food development.

In conclusion, the study demonstrates that EEBS is genotoxicologically harmless under microbial testing model. In addition, EEBS does not present an acute lethal effect at a maximal dose of 20.00 g/kg of body weight on mice. Sub-acute toxicity, confirmed by oral administration at 10 g/kg of body weight for 30 consecutive days, is generally considered safe on rats. Overall, the bamboo shavings extract is of low toxicity and EEBS is promising in functional food development.

Abbreviations

2-AF, 2-Aminofluorene; ALT, aminine aminotransferase; AST, aspartate aminotransferase; BLC, basophil leukocyte count; BUN, blood urea nitrogen; CPA, cyclophosphamide; CRE, creatinine; DAD, diode array detector; 1,8-DAA, 1,8-dihydroxyanthraquinone; EBS, extract of bamboo shaving; EEBS, ethanol extract of bamboo shavings; ELC, eosinophil leukocyte count; GLP, Good Laboratory Practice; Glu, glucose; HGB, hemoglobin; ICR-191, acridine mutagen; LLC, lymph leukocyte count; MLC, mononuclear leukocyte count; MMS, methyl methanesulphonate; MN, frequencies of micronucleus; MTD, maximum tolerated dose; MNRETS, Micronuclei peripheral reticulocytes; NCE, normal chromatic erythrocytes; NLC, neutrophil leukocyte count; NOAEL, no-observed-adverse-effect level; PA, phenolic acids; PCE, polychromatic erythrocytes; P-NQ, P-nitroquinoline; RBC, red blood cell count; SFE, supercritical fluid extraction technique; TC, total cholesterol; TF, total flavonoids; TP, total protein; TG, triglycerides; WBC, white blood cell count.

REFERENCES

- Abdel-Wahab MH, El-Mahdy MA, Abd-Allah MF, Helal GK, Khalifa F, Hamada FM (2003). Influence of *p*-coumaric acid on doxorubicin-induced oxidative stress in rat's heart. *Pharmacol. Res.* 48: 461-465
- Al-Fayez M, Cai H, Tunstall R, Steward WP, Gescher AJ (2006). Differential modulation of cyclooxygenase-mediated prostaglandin production by the putative cancer chemopreventive flavonoids tricetin, apigenin and quercetin. *Cancer Chemother. Pharmacol.* 58: 816-825.
- Ames BN, McCann J, Yamasaki E (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. *Mutat. Res.* 31: 347-364.
- Cai H, Hudson EA, Mann P, Verschoyle RD, Greaves P, Manson MM, Steward WP, Gescher AJ (2004). Growth inhibitory and cell cycle-arresting properties of the rice bran constituent tricetin in human-derived breast cancer cells in vitro and in nude mice in vivo. *Br. J. Cancer.* 91: 1364-1371.
- Cai H, Al-Fayez M, Tunstall RG, Platton S, Greaves P, Steward WP, Gescher AJ (2005). The rice bran constituent tricetin potently inhibits cyclooxygenase enzymes and interferes with intestinal carcinogenesis in *ApcMin* mice. *Mol. Cancer Ther.* 4: 1287-1292.

- Ferguson LR, Zhu ST, Harris PJ (2005). Antioxidant and antigenotoxic effects of plant cell wall hydroxycinnamic acids in cultured HT-29 cells. *Mol. Nutr. Food Res.* 49: 585-693.
- Guglielmi F, Luceri C, Giovannelli L, Dolara P, Lodovici M (2003). Effect of 4-coumaric and 3, 4-dihydroxybenzoic acid on oxidative DNA damage in rat colonic mucosa. *Br. J. Nutr.* 89(5): 581-587
- Hayashi M, Morita T, Komada Y, Sofuni T, Ishidate M (1990). The micronucleus assay with mouse peripheral blood reticulocytes using acridine orange-coated slides. *Mutat. Res.* 245: 245-249.
- Hudson EA, Dinh PA, Kokubun T, Simmonds MSJ, Gescher A (2000). Characterization of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. *Cancer Epidemiol. Biomarkers Prev.* 9: 1163-1170.
- Hu C, Zhang Y, Kitts DD (2000). Evaluation of antioxidant and prooxidant activities of bamboo *Phyllostachys nigra* var. Henonis leaf extract in vitro. *J. Agric. Food Chem.* 48(8): 3170-3176.
- Jiao JJ, Zhang Y, Lou DD, Wu XQ, Zhang Y (2007a). Antihyperlipidemic and antihypertensive effect of a triterpenoid-rich extract from bamboo shavings and vasodilator effect of friedelin on phenylephrine-induced vasoconstriction in thoracic aortas of rats. *Phytother. Res.* 21:1135-1141.
- Jiao JJ, Zhang Y, Liu CM, Liu JE, Wu XQ, Zhang Y (2007b). Separation and purification of triclin from an antioxidant product derived from bamboo leaves. *J. Agric. Food Chem.* 55:10086-10092.
- Khan N, Mukhtar H (2007). Tea polyphenols for health promotion. *Life Sci.* 81(7): 519-533.
- Luceri C, Giannini L, Lodovici M, Antonucci E, Abbate R, Masini E, Dolara P (2007). *p*-Coumaric acid, a common dietary phenol, inhibits platelet activity in vitro and in vivo. *Br. J. Nutr.* 97: 458-463
- Lu BY, Wu XQ, Tie XW, Zhang Y, Zhang Y (2005). Toxicology and safety of anti-oxidant of bamboo leaves. Part 1: Acute and subchronic toxicity studies on anti-oxidant of bamboo leaves. *Food Chem. Toxicol.* 43: 783-792.
- Lu BY, Wu XQ, Shi JY, Dong YJ, Zhang Y (2006). Toxicology and safety of antioxidant of bamboo leaves. Part 2: Developmental toxicity test in rats with antioxidant of bamboo leaves. *Food Chem. Toxicol.* 44: 1739-1743.
- Maron DM, Ames BN (1983). Revised methods for the Salmonella mutagenicity test. *Mutat. Res.* 113: 173-215.
- Ministry of Health PR China (2003). Technical standards for testing and assessment of health food. Beijing, pp. 203-204.
- Shoji T, Akazome Y, Kanda T, Ikeda M (2004). The toxicology and safety of apple polyphenol extract. *Food Chem. Toxicol.* 42: 959-967.
- Yao XB, Wu XQ, Zhang Y (2004). Quantitative analysis of triterpenoid friedelin in bamboo bark (Zhuru) by GC. *Chin. J. Pharm. Anal.* 24: 387-390.
- Yusuf N, Irby C, Katiyar SK, Elmets CA (2007). Photoprotective effects of green tea polyphenols. *Photodermatol. Phot.* 23(1): 48-56.
- Wyrobek AJ, Bruce WR (1975). Chemical induction of sperm abnormalities in mice. *Proc. Natl. Acad. Sci. USA.* 72: 4425-4429.
- Zern TL, Fernandez ML (2005). Cardioprotective effects of dietary polyphenols. *J. Nutr.* 135(10): 2291-2294.
- Zhang Y (1995). Studies of the functional factors in bamboo leaves. Ph.D. thesis of Wuxi University of Light Industry, pp. 52-53.
- Zhang Y, Wu XQ, Yu ZY (2002). Activity of the leaves of bamboo, *phyllostachys nigra*, and ginkgo bilabo. *China J. Chin. Meteria Medica.* 27(4): 254-257.
- Zhang Y, Wu XQ, Ren YP, Fu JY, Zhang Y (2004). Safety evaluation of a triterpenoid-rich extract from bamboo shavings. *Food Chem. Toxicol.* 42: 1867-1875.
- Zhang Y, Yao XB, Bao BL, Zhang Y (2006). Anti-fatigue activity of a triterpenoid-rich extract from Chinese bamboo shavings (*Caulis bambusae* in taeniam). *Phytother. Res.* 20: 872-876.
- Zhang Y, Tie XW, Bao BL, Wu XQ, Zhang Y (2007). Metabolism of flavone C-glucosides and *p*-coumaric acid from antioxidant of bamboo leaves (AOB) in rats. *Br. J. Nutr.* 97(3): 484-494.