JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Protective Effects of Bilberry (*Vaccinium myrtillus* L.) Extract on $KBrO_3$-Induced Kidney Damage in Mice

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Potassium bromate ($KBrO_3$) is an oxidizing agent used as a food additive which causes kidney damage as a potent nephrotoxic agent, and the mechanism may be explained by the generation of oxygen free radicals. Our experiments showed that single intraperitoneal administration of 200 mg/kg $KBrO_3$ could induce serious kidney damage, with an increase in serum blood urea nitrogen (BUN) and creatinine levels. Five-day oral administration of bilberry (*Vaccinium myrtillus* L.) extract at 50, 100, and 200 mg/kg resulted in a reversal in serum BUN and creatinine to normal levels and decreased kidney malondialdehyde (MDA), nitric oxide (NO), and xanthine oxidase (XOD) levels. Also, bilberry extract improved oxygen radical absorbance capacity (ORAC) levels in kidney tissue, which showed that bilberry extract reduced the degree of oxidative stress and kidney damage induced by $KBrO_3$. These findings demonstrate that the protective effect of bilberry extract is attributed to its free radical scavenging activity and lipid peroxidation inhibitory effect.

KEYWORDS: Bilberry (*Vaccinium myrtillus* L.) extract; anthocyanins; potassium bromate ($KBrO_3$); kidney damage; oxidative stress

INTRODUCTION

Potassium bromate ($KBrO_3$) is an oxidizing agent found in drinking water as a disinfection byproduct of surface water ozonation and has been used as a food additive such as in the bread-making process (*1*). However, $KBrO_3$ can cause clinical intoxications including severe and irreversible sensorineural hearing loss and adverse effects on the vestibuloocular reflex system (*2–5*). Recently, it was reported as a carcinogen, which induces chromosome aberration and 8-hydroxydeoxyguanosine generation and is capable of initiating and promoting renal tumorigenesis (*6–8*). The mechanism of $KBrO_3$-induced kidney damage might be explained on the basis of elevation in $ONOO^-$ level, reactive oxygen species, and 8-hydroxydeoxyguanosine levels in renal DNA induced by oxidative stress (*9, 10*).

Bilberry (*Vaccinium myrtillus* L.), a native of Europe and North America, is a low-growing ericaceous dwarf shrub that can be commonly found in the herbaceous layer of boreal forests. As reported, bilberry extract has the ability to improve blood vessel conditions in the retina and helps to increase night visual acuities (*11*), as well as to treat microcirculation disease and maintain normal vascular permeability (*12*). Its potent physiological functions can be correlated to its high content of anthocyanin pigments.

Anthocyanins are naturally occurring water-soluble glycoside flavonoid pigments with a flavylium cation structure. There are more than 250 naturally existing anthocyanins which consist of one of six aglycons glycosylated with various sugar substitutes. The major aglycons are delphinidin, cyanidin, pelargonidin, petunidin, peonidin, and malvidin. Anthocyanin components show high *in vitro* and *in vivo* antioxidative capacity and also inhibit low-density lipoprotein (LDL) oxidation. They have also been shown to have vasoprotective antiinflammatory activity (*12*) and play an important role as radical scavengers, inhibit NO production, attenuate oxidative stress, and contribute to maintaining normal physiological functions (*12–15*). Since bilberry extract has protective effects in various pathophysiological conditions via its antioxidative activities, we speculate that bilberry extract might inhibit $KBrO_3$-induced kidney damage. Therefore, we investigated the prophylactic effect of bilberry extract in mice subjected to $KBrO_3$-induced oxidative stress and subsequent kidney damage.

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10.1021/jf072640s CCC: $40.75
Published on Web 12/20/2007

MATERIALS AND METHODS

Materials and Chemicals. Bilberry extract was purchased from Indena S.P.A. (Milan Italy). Authentic standards of malvidin-3-*O*-gal, malvidin-3-*O*-glu, cyanidin-3-*O*-gal, and cyanidin-3-*O*-glu were obtained from Extrasynthese S.A. (Genay, France), Vitamin C was purchased from Sigma Chemical Co. Xanthine oxidase (XOD) kits, blood urea nitrogen (BUN) kits, malondialdehyde (MDA) kits, and albumin quantitation kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). Creatinine was purchased from Sinopharm Chemical Co. (Shanghai, China). 2,2-Azobis(2-amidinopropane) dihydrochloride (AAPH), sodium fluorescein (FL), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, a water-soluble vitamin E analogue) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Measurement of Anthocyanin Content in Bilberry Extract. Anthocyanins in bilberry extract were qualitatively and quantitatively analyzed employing the method described by Dugo (*16*) and Baj (*17*). The analysis was performed with a DIONEX system, a P680 HPLC pump, and a PDA-100 photodiode array detector at 535 nm. Each anthocyanin was quantitated by measuring against a cyanidin-3-*O*-glu standard calibration curve. HPLC-MS analysis was carried out on an Agilent series 1100 HPLC system equipped with UV–vis detector and coupled online with a Bruker Esquire 2000 mass spectrometer (Bruker Co., Switzerland). Electrospray ionization (ESI) was performed in positive ion mode, and the scan range was from 150 to1500 amu.

Malvidin-3-*O*-gal, malvidin-3-*O*-glu, cyanidin-3-*O*-gal, cyanidin-3-*O*-glu, and bilberry extract were dissolved at 0.50 mg/mL in 10% HCOOH before being appropriately diluted with the same solution and then filtered through a 0.45 μm poly disk filter. Each 20 μL sample was eluted through a Waters RP-18 column (4.6 × 250 mm) at 30 °C. The mobile phase composed of (A) water/formic acid (90:10 v/v) and (B) methanol/acetonitrile/water/formic acid (22.5:22.5:40:10 v/v) was at a flow rate of 1.0 mL/min using a gradient program (solvent B was increased linearly, following an initial hold of 45 min, from 9% to 35% in the next 45 min).

Animals and Treatment. Seven-week-old male KM mice were purchased from the Center of Laboratory Animal Science Research of Southern Medical University, Guangzhou, China. All mice were kept in a specific pathogen-free animal room under the controlled condition of temperature (23 ± 1 °C) and lighting (12 h dark−light cycle) and were provided with standard laboratory diet and tap water. The animals were allowed to acclimate to the environment for 1 week before the experiment.

In the present study, mice were randomly divided into six groups with 10 animals each. Groups I and II were fed saline once daily for 5 days at a dose of 0.1 mL/10 g body weight. Groups III, IV, and V were given an oral administration of bilberry extract once daily for 5 days at dosages of 50, 100, and 200 mg/kg. Group VI received an oral administration of vitamin C once daily for 5 days at a dose of 200 mg/kg. Following the final treatment with bilberry extract, the animals of groups II−IV received a single intraperitoneal injection of $KBrO_3$ at 200 mg/kg (*18*), whereas group I received an intraperitoneal injection of saline (0.1 mL/10 g). Six hours after the $KBrO_3$ injection, the mice were sacrificed under ether anesthesia.

Preparation of Serum, Tissue Samples, and Measurement of Protein Contents. Under ether anesthesia, blood was collected in test tubes following cardiac puncture, and kidneys were quickly removed. After 30 min of standing, blood samples were centrifuged at 5000 rpm for 10 min at 4 °C by refrigerated centrifuge (Sigma Co., Germany) to obtain the serum and acidified with 6% perchloric acid to precipitate protein. Next, the samples were centrifuged at 12000 rpm for 15 min at 4 °C; the supernatant was filtered through a 0.45 μm poly disk prior to HPLC analysis. Kidney samples were homogenized in chilled potassium phosphate buffer (pH 7.4) using an ULTRA-TURRAX T8 homogenizer (GmbH Co., Germany) and centrifuged at 10000 rpm for 10 min at 4 °C. A 2% kidney homogenate was used to determine the protein concentration using a Coomassie brilliant blue kit with bovine serum albumin as the standard.

Measurement of Blood Urea Nitrogen (BUN) Levels. Serum samples were assayed for BUN levels with BUN kits according to the diacetyl monoxime method. Following heating with acid, urea nitrogen in serum reacts with diacetyl to produce pink-colored diazine and was measured at 520 nm with a MK_3 microplate reader (Labsystems Co., Finland) and expressed in milligrams per liter.

Measurement of Serum Creatinine Levels. Serum creatinine concentration was assayed by HPLC (Hitachi, Japan) as described by Jen (*19*). The following chromatographic conditions were applied: a Cosmosil 5C18 column (4.6 × 150 mm; Nacalai Tesque, Japan) at room temperature (25 °C), potassium phosphate buffer solution (0.02 M, pH 6.5) at a flow rate of 1.0 mL/min, and UV detection at 235 nm.

Measurement of XOD Levels in Kidney Tissues. The XOD level in kidney tissue was determined using a commercial XOD kit. XOD in the sample catalyzed the oxidation of hypoxanthine to xanthine to produce the superoxide radical which eventually results in the pink-colored adduct detected at 530 nm with a MK_3 microplate reader (Labsystems Co., Finland).

Measurement of MDA Levels in Kidney Tissues. The MDA level in kidney tissue was measured with a commercial MDA kit. In acidic medium, MDA reacted with thiobarbituric acid (TBA) upon boiling, and the pink-colored MDA−TBA adduct was detected at 532 nm with a MK_3 microplate reader (Labsystems Co., Finland).

Measurement of Oxygen Radical Absorbance Capacity (ORAC) Levels in Kidney Tissues. The peroxyl radical scavenging activity of kidney tissue was assayed on the basis of the protocol described by Davalos (*20*). Peroxyl radicals were generated at a controlled rate by thermal decomposition of AAPH. The 180 μL assay solution contained 20 μL of kidney homogenate and 20 μL of 75 mM potassium phosphate buffer with 140 μL of AAPH solution (final concentration 12.8 mmol/L). The reaction was started by adding 20 μL of fluorescein (final concentration 63 nmol/L) to the assay solution. Decay of the fluorescein signal was determined at 485/527 nm in a GENios Lueifcrase microplate reader (TECAN, Switzerland) at 37 °C.

Measurement of NO Levels in Kidney Tissues. The NO levels were determined by the Griess method (*21*). A 40 μL sample was transferred into 96-well microplates, and 160 μL of Griess reagent (1% sulfanilamide, 0.1% *N*-(1-naphthyl)ethylenediamine hydrochloride, 2.5% H_3PO_4) was added at room temperature. After 20 min, the purple-azo-dye product was detected at 540 nm with a MK_3 microplate reader (Labsystems Co., Finland).

Measurement of Antioxidative Capacity *in Vitro*. We used the oxygen radical absorbance capacity (ORAC) assay to evaluate the antioxidant properties of the bilberry extract. The assay was carried out on a GENios Lueifcrase microplate reader (TECAN, Switzerland) with fluorescent filters (excitation wavelength, 485 nm; emission wavelength, 527 nm), as in the above-mentioned method. For the *in vitro* experiments, the bilberry extract was dissolved in the potassium phosphate buffer before use.

Statistical Analysis. The data were presented as the mean ± SE. Statistical analysis of data was performed using the SPSS 13.0 statistical package program for windows. One-way analysis of variance (ANOVA) was applied to analyze for difference in data of biochemical parameters among the different groups followed by Dunnett's significant post-hoc test for pairwise multiple comparisons. Differences were considered as statistically significant at the $p < 0.05$ level.

RESULTS

Characterization and Measurement of Anthocyanins in Bilberry Extract. In the HPLC analysis, the 15 anthocyanin peaks (**Figure 1** and **Table 1**) were identified by comparing the masses and referred to the findings reported by Baj (*17*) and Cooke (*22*). Each gram of bilberry extract contained 420.33 mg of anthocyanins expressed as cyanidin 3-*O*-glucoside.

Effect of Bilberry Extract on Serum BUN and Creatinine Levels. The present finding is the first report on the effect of anthocyanin-enriched bilberry extract (42.03% of anthocyanins) on serum BUN and creatinine levels in mice treated with $KBrO_3$. As shown in **Table 2**, the levels of BUN and creatinine were significantly higher in the $KBrO_3$-treated animals when compared with normal mice (247.31 ± 3.22 mg/L versus 215.10 ±

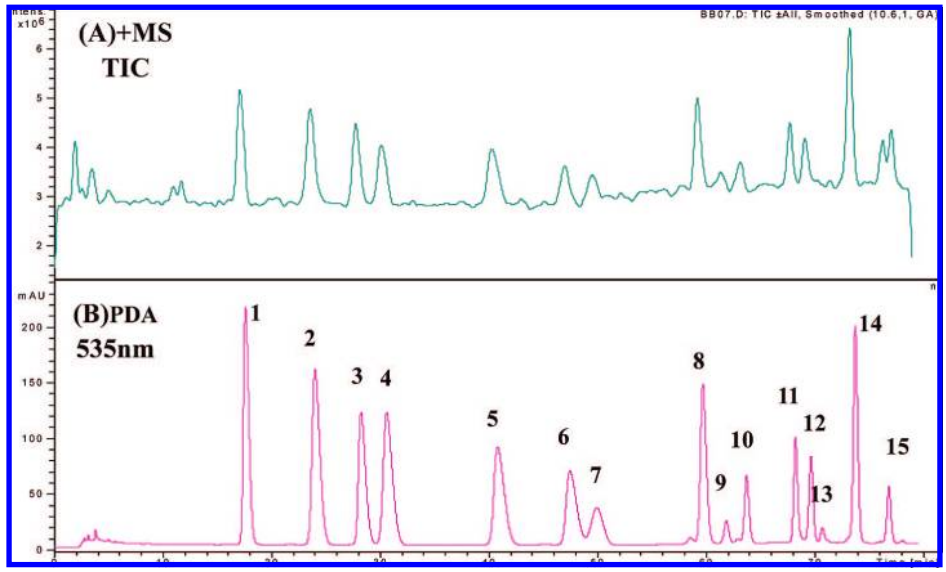

Figure 1. HPLC-MS analysis of anthocyanins in bilberry extract. (**A**) HPLC-MS total ion chromatogram in positive ion mode under experimental conditions. (**B**) Photodiode array chromatogram at 535 nm under experimental conditions. The identity of each anthocyanin is stated in **Table 1**.

Table 1. Amount of 15 Anthocyanins in Bilberry Extract

		amount (cyanidin-3-*O*-glu equivalent)	
no.	anthocyanin	mg/g	%
1	delphinidin-3-*O*-gal	49.07	4.91
2	delphinidin-3-*O*-glu	54.21	5.42
3	cyanidin-3-*O*-gal[a]	40.03	4.00
4	delphinidin-3-*O*-ara	49.06	4.91
5	cyanidin-3-*O*-glu[a]	41.81	4.18
6	cyanidin-3-*O*-ara	32.18	3.22
7	petunidin-3-*O*-gal	17.92	1.79
8	petunidin-3-*O*-glu	38.49	3.85
9	peonidin-3-*O*-gal	4.34	0.43
10	petunidin-3-*O*-ara	13.44	1.34
11	peonidin-3-*O*-glu	15.90	1.59
12	malvidin-3-*O*-gal[a]	13.23	1.32
13	peonidin-3-*O*-ara	1.75	0.18
14	malvidin-3-*O*-glu[a]	40.15	4.02
15	malvidin-3-*O*-ara	8.75	0.88
	total anthocyanins	420.33	42.03

[a] Compared with authentic standards.

Table 2. Effect of Bilberry Extract on Serum BUN and Creatinine Levels in Mice Treated with $KBrO_3$

treatment[a]	BUN (mg/L)	creatinine (μg/mL)
none	215.10 ± 2.82	9.91 ± 0.86
$KBrO_3$	247.31 ± 3.22[b]	22.26 ± 1.75[b]
$KBrO_3$ + Vc (200 mg/kg)	225.93 ± 1.84[c]	13.33 ± 1.09[d]
$KBrO_3$ + BE (200 mg/kg)	146.25 ± 3.56[d]	12.00 ± 0.64[d]
$KBrO_3$ + BE (100 mg/kg)	185.94 ± 3.64[d]	13.10 ± 0.56[d]
$KBrO_3$ + BE (50 mg/kg)	210.25 ± 3.50[d]	16.22 ± 1.14[d]

[a] None = normal control; BE = bilberry extract. KM mice were administered intraperitoneally with 200 mg/kg $KBrO_3$. The results represent the mean ± SE obtained from 10 animals in each group. [b] Significantly different from normal mice at $p < 0.001$. [c] Significantly different from $KBrO_3$-treated mice at $p < 0.01$ (one-way ANOVA followed by Dunnett's test). [d] Significantly different from $KBrO_3$-treated mice at $p < 0.001$ (one-way ANOVA followed by Dunnett's test).

2.82 mg/L; 22.26 ± 1.75 μg/mL versus 9.91 ± 0.86 μg/mL, respectively). Five day oral intake of bilberry extract remarkably reduced the levels of BUN and serum creatinine ($p < 0.01$) in treated mice. A dose−response relationship was observed, indicating that bilberry extract attenuated increased levels of BUN and creatinine caused by $KBrO_3$.

Table 3. Effect of Bilberry Extract on XOD and MDA Levels in Kidney of Mice Treated with $KBrO_3$

treatment[a]	XOD (units/g of protein)	MDA (nmol/mg of protein)
none	28.18 ± 1.12	41.52 ± 1.92
$KBrO_3$	36.45 ± 2.08[b]	62.47 ± 3.16[c]
$KBrO_3$ + Vc (200 mg/kg)	30.19 ± 1.37[d]	50.15 ± 3.12[d]
$KBrO_3$ + BE (200 mg/kg)	26.65 ± 0.66[f]	43.24 ± 1.75[f]
$KBrO_3$ + BE (100 mg/kg)	28.70 ± 0.63[e]	48.38 ± 3.02[e]
$KBrO_3$ + BE (50 mg/kg)	30.15 ± 0.90[d]	52.14 ± 1.92[d]

[a] None = normal control; BE = bilberry extract. KM mice were administered intraperitoneally with 200 mg/kg $KBrO_3$. The results represent the mean ± SE obtained from 10 animals in each group. [b] Significant difference compared with normal mice at $p < 0.01$. [c] Significant difference compared with normal mice at $p < 0.001$. [d] Significant difference compared with $KBrO_3$-treated mice at $p < 0.05$ (one-way ANOVA followed by Dunnett's test). [e] Significant difference compared with $KBrO_3$-treated mice at $p < 0.01$ (one-way ANOVA followed by Dunnett's test). [f] Significant difference compared with $KBrO_3$-treated mice at $p < 0.001$ (one-way ANOVA followed by Dunnett's test).

Effect of Bilberry Extract on XOD and MDA Levels in Kidney Tissues. As seen in **Table 3**, XOD levels were found to be significantly increased in $KBrO_3$-treated mice than those in normal mice ($p < 0.01$). A dose-dependent suppression of XOD levels in kidney tissues was observed in mice treated with bilberry extract. Similarly, vitamin C also reduced the XOD levels significantly when compared with the $KBrO_3$-treated group.

Lipid peroxidation product, MDA in kidney tissues, increased by 150% following treatment with $KBrO_3$ (**Table 3**). Treatment of the animals with bilberry extract and vitamin C significantly reduced the high level of MDA in the kidney tissue. At 100 and 200 mg/kg, bilberry extract could suppress and reverse XOD and MDA to levels comparable to normal mice.

Effect of Bilberry Extract on ORAC and NO Levels in Kidney Tissues. Table 4 shows the effect of bilberry extract on $KBrO_3$-mediated ORAC levels during oxidative stress caused by $KBrO_3$. Treatment with $KBrO_3$ alone resulted in the reduction of the ORAC levels to 67% of the normal mice. The results suggest that oxidative stress plays an important role in $KBrO_3$-induced kidney damage. By contrast, bilberry extract at 100 and 200 mg/kg significantly promoted the recovery of ORAC levels in a dose-dependent manner to levels similar to normal

Table 4. Effect of Bilberry Extract on ORAC and NO Levels in the Kidney of Mice Treated with $KBrO_3$

treatment[a]	ORAC (μM Trolox equiv)	NO (μmol/mL)
none	23.69 ± 0.65	4.03 ± 0.27
$KBrO_3$	15.87 ± 1.23[c]	5.61 ± 0.44[b]
$KBrO_3$ + Vc (200 mg/kg)	22.96 ± 1.93[d]	3.39 ± 0.39[d]
$KBrO_3$ + BE (200 mg/kg)	26.02 ± 1.83[e]	3.24 ± 0.34[e]
$KBrO_3$ + BE (100 mg/kg)	21.99 ± 1.08[d]	4.42 ± 0.53
$KBrO_3$ + BE (50 mg/kg)	20.38 ± 1.79	5.21 ± 0.29

[a] None = normal control; BE = bilberry extract. KM mice were administered intraperitoneally with 200 mg/kg $KBrO_3$. The results represent the mean ± SE obtained from 10 animals in each group. [b] Significant difference compared with normal mice at $p < 0.01$. [c] Significant difference compared with normal mice at $p < 0.001$. [d] Significant difference compared with $KBrO_3$-treated mice at $p < 0.01$ (one-way ANOVA followed by Dunnett's test). [e] Significant difference compared with $KBrO_3$-treated mice at $p < 0.001$ (one-way ANOVA followed by Dunnett's test).

animals (26.02 ± 1.83 and 21.99 ± 1.08 μM Trolox equiv versus 23.69 ± 0.65 μM Trolox equiv, respectively).

As shown in **Table 4**, the kidney level of NO was distinctively higher in the $KBrO_3$-treated animals when compared with the normal mice (4.03 ± 0.27 μmol/mL versus 5.61 ± 0.44 μmol/mL). Treatment of the animals with 200 mg/kg bilberry extract and vitamin C significantly reduced the high level of NO in kidney tissues.

Antioxidative Capacity of Bilberry Extract *in Vitro*. **Figure 2** shows the working curves of fluorescein oxidation used as an index of resistance time for the oxidative reaction. The linear relationship between the net area and different concentrations of the antioxidant was evaluated. **Figure 2A**,**B** represents the trolox and vitamin C, a single antioxidant, and fluorescence decay curves, respectively. A linear regression curve of four concentrations versus the net area under the curve was obtained. Trolox increased the level of scavenging activity for the oxidation of fluorescein in a dose-dependent manner. In **Figure 2C**, quenching curves of disodium fluorescein illustrate the ability of bilberry extract to absorb the peroxyl radical as compared with that of the standard Trolox. The four concentrations of bilberry extract gave a good fit to the linear regression relationship. As seen in **Figure 2D**, the antioxidative capacity of bilberry extract was stronger than that of vitamin C.

DISCUSSION

In the present experiments, the serum markers of kidney damage, serum BUN, and creatinine levels were elevated in $KBrO_3$-treated mice. Previous studies reported that oxidative stress plays an important role in the pathophysiology of $KBrO_3$-mediated kidney damage (*9*). Exposure to $KBrO_3$ leads to an increase in kidney XOD and MDA levels. Increased XOD catalyzes the oxidation of hypoxanthine to xanthine and produces free radicals, which further generates superoxide anions under pathology condition. Hence, increased XOD levels seem to accelerate the potassium bromate-induced acute kidney damage. MDA, the final metabolite of lipid peroxidation, which is utilized as an available parameter of oxidative stress, not only translates reactive oxygen species into active chemicals but also magnifies the function of reactive oxygen species through the chain reaction, inducing cellular metabolism and functional impairment (*23*). In addition, the basal level of NO is important to sustain the bloodstream and glomerular filtration rate in kidney. Excess NO reacts with the oxygen free radical, producing cytotoxic radical $ONOO^-$, which may damage normal cellular function. In the current study, the remarkable elevation in kidney tissue NO level by 39% further contributed to $KBrO_3$-mediated oxidative stress and kidney damage.

Although the detailed mechanism of $KBrO_3$-mediated kidney damage is still unknown, previous studies found that $KBrO_3$-induced oxidative stress can cause free radical reactions to produce deleterious modifications in membranes, proteins, enzymes, and DNA, which may contribute to kidney damage (*24*). Therefore, it is important to find effective scavengers of active oxygen radicals. Resveratrol, melatonin,

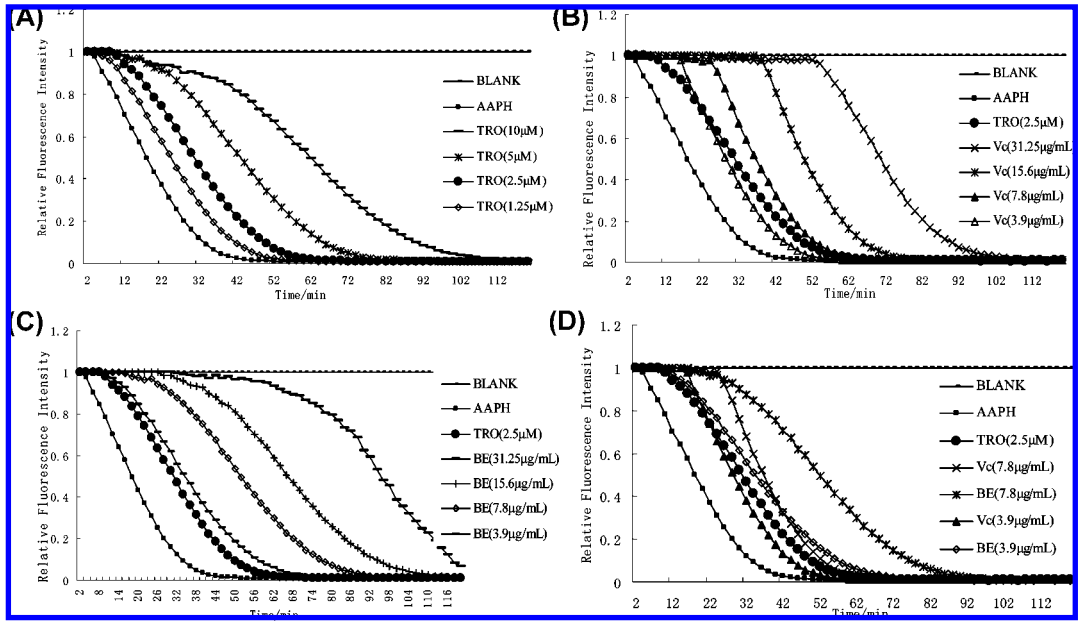

Figure 2. Scavenging activity of bilberry extract (BE) and vitamin C against fluorescence decay induced with AAPH. Curves of fluorescence decay induced by AAPH as a peroxyl radical generator in the presence of Trolox (**A**), vitamin C (**B**), or bilberry extract (**C**) at different concentrations are shown. (**D**) represents the comparison of fluorescence decay curves between bilberry extract and vitamin C. Trolox, a water-soluble vitamin E analogue, was used as a control standard. The antioxidative activity of a sample is expressed as the net area under the curve. Data are expressed as the means of three experiments.

and vitamin E and the spin trapping agent α-phenyl-*N*-*tert*-butyl nitrone (PBN) have been reported to protect against oxidative damage to kidney DNA (*25*). Khan et al. reported that soy isoflavones are effective in improving kidney function in $KBrO_3$-treated rats (*26*).

Bilberry is well documented in scientific literature as an antioxidant with various pharmacological actions. However, only a few reports have explored the protective effects of bilberry extract on kidney damage. In this study, we found that bilberry extract is effective in attenuating $KBrO_3$-mediated kidney damage as reflected by reduction in serum BUN and creatinine and also ameliorating the oxidative parameters, viz., MDA, XOD, and NO, as well as the ORAC level. The observed protective effect of the bilberry extract against oxidative damage induced by $KBrO_3$ of mice is probably due to the antioxidative properties of its constituents, mainly anthocyanins. The antioxidative activities of various bilberry extracts and their anthocyanins have been previously found: a bilberry extract containing 25% anthocyanins displayed cytoprotective effects against oxidative damage of rat hepatocytes induced by *tert*-butyl hydroperoxide and allyl alcohol (*27*) and was regarded as the most effective in inhibiting copper-induced protein and lipid oxidation in a lactalbumin−liposome oxidation system (*28*). Nakajima has reported that bilberry extract (28.8% of anthocyanins) scavenged 1,1-diphenyl-2-picrylhydrazyl (DPPH) with an IC_{50} (50% inhibitory concentration) of 40 μg/mL in 2004 (*29*).

Our analysis results indicated that the total anthocyanin content is 420.33 mg/g of bilberry extract. Therefore, bilberry extract can be considered a better source of antioxidants. Next, we also estimated antioxidative activities of bilberry extract *in vitro* by the ORAC method. As shown in **Figure 2C**, the bilberry extract scavenged the free radicals generated by AAPH in a dose-dependent manner. A comparison of bilberry extract and vitamin C with 7.8 and 3.9 μg/mL (**Figure 2D**) showed that bilberry extract is a more powerful radical scavenger than vitamin C. The same effect was observed in *in vivo* experiments; the protective effects of 200 mg/kg bilberry extract were comparable or even stronger than that of 200 mg/kg vitamin C. We intend to investigate the mechanisms of anthocyanin pharmacological action in the near future.

We conclude that the protective effects of anthocyanin-enriched bilberry extract on $KBrO_3$-induced kidney damage is due to an increased antioxidative capacity of kidney tissue via a reduction in XOD, MDA, and NO levels and improved ORAC level. These results warrant further investigation into the pharmacology of bilberry extract and its major anthocyanins under physiological oxidative stress conditions. Further research could contribute to the discovery of a bioactive phytochemical that ameliorates physiological dysfunction due to lifestyle-related diseases such as hypertension and metabolic disorders.

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Received for review September 6, 2007. Revised manuscript received November 20, 2007. Accepted November 21, 2007.

JF072640S