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Blackberry Extract Attenuates Oxidative Stress through Up-regulation of Nrf2-Dependent Antioxidant Enzymes in Carbon Tetrachloride-Treated Rats

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Supporting Information

ABSTRACT: The aim of this study was to investigate the protective ability of blackberry extract (BE) against oxidative stress in carbon tetrachloride (CCl_4)-treated rats. The results showed that treatment with BE attenuated lipid peroxidation that was increased by CCl_4 and also markedly recovered the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR), that were decreased by CCl_4. BE also elevated the protein expression levels of NF-E2-related factor-2 (Nrf2), CuZnSOD, MnSOD, GPx-1/2, and heme oxygenase-1 (HO-1), but not that of catalase. Furthermore, the administration of BE significantly attenuated the levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) that were increased by CCl_4. Therefore, the present study suggests that BE possesses significant protective effects against in vivo oxidative stress.

KEYWORDS: blackberry, carbon tetrachloride, oxidative stress, antioxidant enzyme, lipid peroxidation, NF-E2-related factor-2 (Nrf2)

INTRODUCTION

Reactive oxygen species (ROS), such as superoxide radical, hydrogen peroxide, and hydroxyl radical, are induced by a variety of factors, including xenobiotics, drugs, heavy metals, ultraviolet light (UV), and ionizing radiation. Although ROS have an important role in normal physiological conditions, high levels of ROS cause damage to several biomolecules in cells, such as proteins, lipids, and DNA,[1] and have been linked to aging, neurodegenerative diseases, cancer, atherosclerosis, and diabetes.[2] Carbon tetrachloride (CCl_4) has been widely used to investigate liver injury associated with oxidative stress and free radicals. The metabolism of CCl_4 is mediated by cytochrome P450, and trichloromethyl free radical ($^{\bullet}CCl_3$) and trichloromethyl peroxy radical (Cl_3COO^-) are yielded in the liver. These free radicals are highly reactive species that can covalently bind to cellular macromolecules, such as nucleic acids, protein, and lipids, and lead to lipid peroxidation.[3−5] Living organisms have developed several protective mechanisms to prevent ROS formation or to detoxify it. These mechanisms involve antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as nonenzymatic antioxidants, such as glutathione (GSH), uric acid, and vitamins C and E.[6,7]

The blackberry is an excellent source of natural antioxidants and has been used for various medicinal purposes. Recent studies have reported that blackberry extract is able to reduce the risk of cancer and cardiovascular diseases as well as indicate high antioxidant capacity.[8] It has been demonstrated that blackberries contain higher amounts of anthocyanins and other antioxidants, such as flavonols, phenolic acids, ellagic acid, and vitamins C and E.[9] Among these components, the anthocyanins, which are the major polyphenolic compound in blackberries, are excellent scavengers of a wide variety of ROS, such as superoxide radical, hydrogen peroxide, and hydroxyl radical.[10] It has been reported that polyphenolics from blackberries possess antioxidant and anti-inflammatory activities.[11] In addition, several in vitro and in vivo studies have reported that anthocyanins from blackberries have potential biological benefits as antioxidant and/or anti-inflammatory agents.[12,13] Ding et al.[14] have also reported that cyanidin-3-glucoside from blackberries exhibits chemopreventive and chemotherapeutic activity in vivo and in vitro. However, the in vivo antioxidant activity of blackberry extract and their mechanisms were not well understood.

Therefore, in this study, we investigated the protective ability of blackberry extract (BE) and its possible mechanism against in vivo oxidative stress in CCl_4-treated rats.

MATERIALS AND METHODS

Reagents and Antibodies. Analytical grade methanol, ethyl acetate, acetonitrile, and water were purchased from J. T. Baker (Phillipsburg, NJ). Amberlite XAD-7, hydrogen chloride (HCl), trifluoroacetic acid (TFA), CD_3OD, 2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-l-picrylhydrazyl (DPPH), protease inhibitor cocktail, CCl_4, and olive oil were obtained from Sigma Chemical Co. (St. Louis, MO). Silica gel 60 Rp-18 (40−63 lm), Sephadex LH-20 (Amersham Bioscience), and TLC aluminum sheets RP-18 F_{254} were obtained from Merck (Darmstadt, Germany). All chemicals used were of reagent grade and were purchased from Sigma Chemical Co. unless otherwise stated. All solvents were distilled

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before use. Thiobarbituric acid reactive substances (TBARS), SOD, CAT, GPx, and glutathione reductase (GR) assay kits were purchased from Cayman Chemical (Ann Arbor, MI). Antibodies for β-tubulin, Nrf2, HO-1, GPx-1/2, CuZnSOD, MnSOD, and CAT were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The goat anti-rabbit and goat anti-mouse IgG HRP-conjugated antibodies were purchased from Zymed (San Francisco, CA). The Chemiluminescence Detection System was purchased from iNtRON Biotechnology (Seongnam, Korea).

Instruments. NMR spectra were recorded with a JEOL ECX-500 instrument (^{1}H NMR at 500 MHz, ^{13}C NMR at 125 MHz) (JEOL, Tokyo, Japan) in CFCOOD-CD3OD (1:19, v/v) (Sigma-Aldrich Co.). HPLC was performed using an Agilent 1100 series (Boeblingen, Germany) quaternary pump, an Agilent 1100 series diode array detector, and a TSK-GEL ODF-100 V (4.6 × 150 mm, 5 μm; TOSOH, Tokyo, Japan) column. LC-MS was measured in a 3200 Q Trap LC-MS/MS System (Applied Biosystems, Lincoln, NE).

Preparation of Blackberry Extract. Blackberries (new blackberry cultivar Maple mutated from black V3) were obtained from a commercial farm in the region of Wanju (Jeonbuk, Korea) in June 2010. BE was extracted with ethanol (EtOH)/HCl (99:1, v/v) overnight at room temperature and filtered through a membrane filter with a 0.45 μm pore. The filtrate was evaporated for use in this experiment.

Extraction and Isolation of Anthocyanins. Samples weighing 500 g were extracted with 1500 mL of 1% HCl (v/v) in ethanol for 3 days at 4 °C, in darkness. The ethanol extract was concentrated in a rotary evaporator (35 °C) to obtain the crude extract and freeze-dried to powdered form. The concentrated crude extract was purified by partition against ethyl acetate and then subjected to an Amberlite XAD-7 column chromatography (5 × 60 cm). The column was washed with 1500 mL of water, and elution of anthocyanins was conducted with 1500 mL of 1% TFA (v/v) in methanol. The eluate was concentrated under reduced pressure at 25 °C.

Determination of Total Phenolics. Total phenolics (TP) were determined colorimetrically using Folin−Ciocalteu reagent, as described by Julkunen-Tiitto[15] with modifications. A total phenolics assay was conducted by mixing 10 μL of extracts, 10 μL of 2% Na_2CO_3, and 10 μL of 50% Folin−Ciocalteu reagent. Absorbance of the mixture was measured at 725 nm. A standard curve was prepared with gallic acid. Final results were reported as gallic acid equivalents (GAE).

Determination of Anthocyanins. Absorbance of extracts (1 g of the defatted sample was extracted with 1 mL of methanol containing 1% HCl) was measured at 520 and 700 nm in buffers at pH 1.0 and 4.5 and calculated using the following equation: $A = [(A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5}]$ with a molar extinction coefficient of cyanidin-3-glucoside of 26900. Results were expressed as milligrams of cyanidin-3-glucoside equivalent (CGE) per 100 g of dry weight.[16]

Determination of ABTS Radical Scavenging Activity. ABTS radical scavenging measurements were performed according to the method of Lee et al.[17] with modifications described previously.[18] The radical cation was prepared by mixing a 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1:1, v/v) and leaving the mixture for 4−8 h until the reaction was complete and the absorbance was stable. The $ABTS^{\bullet+}$ solution was diluted in ethanol to an absorbance of 0.700 ± 0.05 at 734 nm for measurements. The photometric assay was conducted on 0.9 mL of the $ABTS^{\bullet+}$ solution, and 0.1 mL of extract solution was calculated by determining the decrease in absorbance at different concentrations by using the following equation: $E = [(A_c - A_t)/A_c] \times 100$, where A_t and A_c are the respective absorbance of samples with and without extract solution. The total antioxidant capacities (TAC) were estimated as Trolox equivalent antioxidant capacity (TEAC) interpolation to 50% inhibition ($TEAC_{50}$).

Determination of DPPH Radical Scavenging Activity. Various concentrations of extracted solution were added to 0.15 mM DPPH in EtOH, and the reaction mixture was shaken vigorously.[17] The amount of remaining DPPH radical was determined at 517 nm after 30 min, and the radical-scavenging effect was calculated as follows: $E = [(A_c - A_t)/A_c] \times 100$, where A_t and A_c are the respective absorbances of samples with and without extract solution.

HPLC DAD-MSD Analysis. The anthocyanins from the blackberries were characterized by HPLC diode array detection (DAD) mass spectrometry (MS) analysis. HPLC was performed using an Agilent 1100 series quaternary pump, an Agilent 1100 series diode array detector, and a TSK-GEL ODF-100 V (4.6 × 150 mm, 5 μm; TOSOH) column. BE (20 μL) was injected onto an analytical reverse phase C-18 column (TSK-GEL ODF-100 V, 4.6 × 150 mm, 5 μm; TOSOH). The mobile phase was composed of 10% formic acid in water (A) and acetonitrile (B). The gradient conditions were as follows: 0.5 min, 0% B; 4 min, 9% B; 10 min, 13% B; 20 min, 20% B; 21 min, 100% B, and then held for 10 min before returning to the initial conditions. Other HPLC conditions were as follows: flow rate, 1 mL/min; column temperature, 30 °C; detection, 530 nm; and sample size, 20 μL. The mass spectrometer used was an Agilent mass spectrometer detector (MSD) equipped with an electrospray (MM-ES) ionization source and an ion trap mass analyzer, which was controlled by Chemstation software. The mass parameters were as follows: capillary potential, 4000 V; drying gas temperature, 350 °C; gas flow (N_2), 12 L/min; nebulizer pressure, 35 psig. The instrument was operated in the positive ion mode scanning from m/z 100 to 1000. The mass spectral characteristics of anthocyanins were compared to the cyanidin-3-glucoside standard and with available data in the literature. Quantification was performed by comparison of retention times, and diode array spectra were matched against standards and library spectra.

Animals. Male Sprague−Dawley rats weighing 150 ± 10 g were obtained from Orient Bio Inc. (Seongnam, Korea). They were acclimated for 1 week under standard environmental conditions and fed a standard diet and water ad libitum. The rats were maintained at 23 ± 2 °C on a 12/12 h light−dark cycle in an air-conditioned room. All experimental protocols used in this study were conducted in accordance with the guidelines for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Korea Atomic Energy Research Institute (KAERI-IACUC).

Experimental Design. The 6-week-old rats were randomly divided into six groups with six rats per group as follows: group 1, control; group 2, CCl_4 (1 g/kg) group; group 3, CCl_4 plus BE 100 mg/kg; group 4, CCl_4 plus BE 200 mg/kg; group 5, CCl_4 plus BE 400 mg/kg; and group 6, BE 400 mg/kg only. BE was prepared in normal saline, and 50% CCl_4 was prepared in olive oil. Groups 1 and 2 were administered saline, and groups 3−6 were administered BE orally every other day for 15 days. Three hours after the final administration, groups 1 and 6 were treated intraperitoneally with olive oil. Groups 2−5 were treated intraperitoneally with CCl_4 and fasted for 18 h, and then they were euthanized. Blood was taken from the postcaval vein after the rats had been anesthetized with ether to determine serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. The liver was also quickly collected and used for the antioxidant enzyme assay.

Biochemical Analysis. Superoxide dismutase, catalase, GPx, GR activity, and lipid peroxidation were measured using a commercial kit from Cayman Chemical. Tissue homogenate was prepared according to the manufacturer's protocol. The blood samples collected from each animal were allowed to clot for 30 min at room temperature. The blood serum was centrifuged at 2000g for 15 min at 4 °C. The AST and ALT levels were estimated using a clinical biochemistry automatic analyzer (Hitachi model 7180, Japan).

Western Blotting. The tissues were washed with phosphate-buffered saline (PBS) and homogenized in 10 mL of lysis buffer (50 mM Tris, pH 7.4, 250 mM NaCl, 5 mM EDTA, 1 mM Na_3VO_4, 1% NP40, 0.02% NaN_3, 1 mM PMSF) containing a protease inhibitor cocktail per gram. Samples were lysed for 30 min on ice and centrifuged at 14000g for 15 min at 4 °C.

The protein concentration was then quantified using the Bio-Rad protein assay. The proteins were separated on 12% SDS−polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were then blocked with 5% skim milk in TBS-T (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween 20) for 1 h; then, they were incubated with primary antibodies at 4 °C overnight. The manufacturer's protocol was followed for the dilution of all primary antibodies. The membranes were washed three times with TBS-T for 10 min and incubated with horseradish peroxidase-conjugated secondary antibodies (1:5000) for 2 h at room temperature. They were then washed again, and the blotted proteins were detected using an enhanced chemiluminescence detection system.

Statistical Analysis. All data are presented as the mean ± SD. The significance of differences between the means of the treated and untreated groups was determined by Student's t test. A $p < 0.05$ value was considered to be significant.

RESULTS AND DISCUSSION

Identified Anthocyanins from Blackberries. The blackberries were extracted with acidic EtOH followed by Amberlite XAD-7 column chromatography. The anthocyanins, cyanidin-3-*O*-galactoside (**1**), cyanidin-3-*O*-glucoside (**2**), cyanidin-3-*O*-arabinoside (**3**), cyanidin-3-*O*-xyloside (**4**), cyanidin-3-*O*-malonylglucoside (**5**), cyanidin-3-*O*-dioxalylglucoside (**6**), cyanidin (**7**), cyanidin-3-*O*-methylmalonylglucoside (**8**), cyanidin-3-*O*-methyloxalylglucoside (**9**), and cyanidin-3-*O*-3″,6″-dimalonylglucoside (**10**) were identified, and their chemical structures were elucidated by LC-MS and were confirmed by previous literature[19−23] (Figure 1). (See the Supporting Information for characterization data of compounds **1**−**10**.)

Total Phenol, Total Flavonoids, Total Anthocyanins Content, and Antioxidant Activity. The concentrations of total phenol, flavonoid, and anthocyanin were assayed according to the methods described by Julkunen-Tiitto,[15] Re et al.,[18] and Giusti and Wrolstad.[16] Their content of blackberry extract represented 4199 ± 50, 160 ± 12, and 1669 ± 54 mg/100 g, respectively. The concentration of cyanidin-3-*O*-glucoside in total anthocyanins represented 586 ± 9 mg/100 g (Table 1). The concentrations of total phenol, flavonoid, and anthocyanin quantified in blackberry were higher than previous literature.[24] This result suggests that anthocyanins may be affected by factors including environmental stresses (light, temperature, and agronomic conditions), genetics, and stage of maturity as the results of previous studies.[11] Antioxidant activities of the various methods, such as ABTS and DPPH, have been commonly used to measure the total antioxidative status of various biological specimens.[24] The antioxidant activity of BE measured by the ABTS and DPPH assays indicated 144.3 ± 4.1 (IC_{50}) and 185.9 ± 5.5 (IC_{50}), respectively (Table 1). Sariburun et al.[24] have reported that the antioxidant activity measured by ABTS assay in four blackberry cultivars ranged from 74.92 ± 0.11 to 146.89 ± 4.59 TE/g fresh weight and antioxidant activity measured by DPPH assay ranged from 90.95 ± 1.04 to 177.11 ± 3.17 TE/ g fresh weight. Some studies have also demonstrated that blackberries possess high antioxidant activity as shown in in vitro assays.[11,12,22] Our result is similar to previous studies,[11,12,22,24] suggesting that BE possesses strong antioxidant activity.

Effects of BE on Lipid Peroxidation in CCl_4-Treated Rat Liver. CCl_4 is converted to trichloromethyl free radical ($^{\bullet}CCl_3$) by cytochrome P450, and subsequently, trichloromethyl peroxide (Cl_3COO^-) is formed. These free radicals attack cellular components, such as nucleic acids, proteins, and lipids (especially polyunsaturated fatty acids) and also cause lipid peroxidation. Malondialdehyde (MDA) is an important product of lipid peroxidation. MDA production is commonly used to measure the level of oxidative damage in tissue.[3−5] Therefore, the MDA concentration was measured to evaluate the level of lipid peroxidation

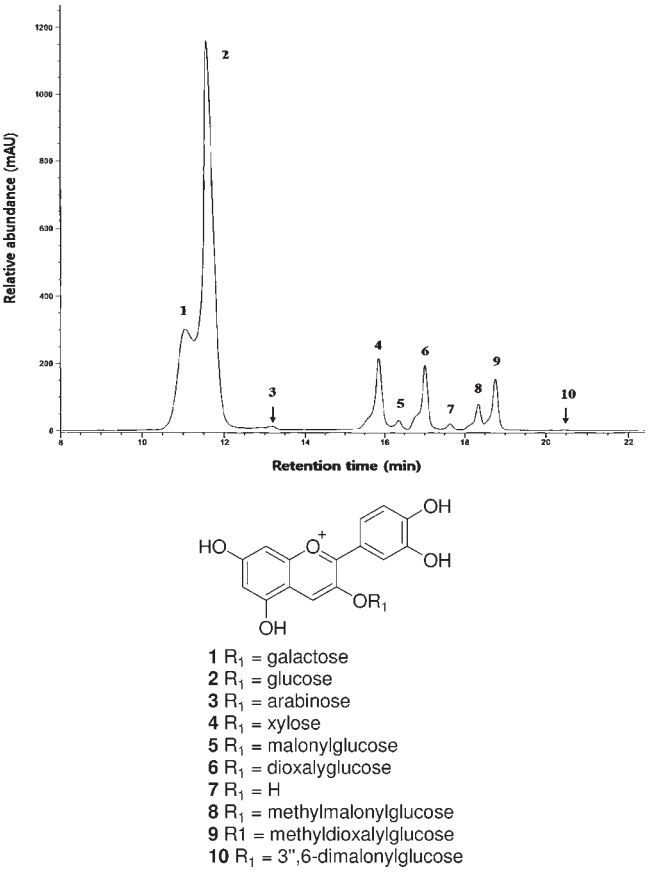

Figure 1. HPLC chromatogram of blackberry extract at 530 nm and structures of the anthocyanins identified from blackberry. Peaks: 1, cyanidin-3-*O*-galactoside; 2, cyanidin-3-*O*-glucoside; 3, cyanidin-3-*O*-arabinoside; 4, cyanidin-3-*O*-xyloside; 5, cyanidin-3-*O*-malonylglucoside; 6, cyanidin-3-*O*-dioxalylglucoside; 7, cyanidin; 8, cyanidin-3-*O*-methylmalonylglucoside; 9, cyanidin-3-*O*-methyldioxalylmalonylglucoside; 10, cyanidin-3-*O*-3″,6″-dimalonylglucoside.

Table 1. Antioxidant Activity and Anthocyanin and Polyphenol Contents of Blackberries[a]

anthocyanin and polyphenol contents (mg/100 g)	
total phenols	4199 ± 50
total flavonoids	160 ± 12
total anthocyanins[b]	1669 ± 54
cyanidin-3-glucoside[c]	586 ± 9
antioxidant activity (mg/L)	
DPPH[d]	185.9 ± 5.5
ABTS[d]	144.3 ± 4.1

[a] All extracts were examined in a set of experiments repeated three times. [b] Total anthocyanin content as determined by molar extinction coefficient. [c] Cyanidin-3-glucoside as characterized using HPLC. [d] Sample concentration (mg/L) was 50% activity loss.

in the liver (Figure 2). Rats treated with CCl_4 alone showed increased MDA levels (146 ± 11%) compared with the control group (100 ± 18%). However, pretreatment with BE decreased the MDA levels in the rats treated with CCl_4 in a dose-dependent manner. Thus, this result suggests that BE is able to attenuate oxidative stress through the inhibition of CCl_4-induced lipid peroxidation in rat liver.

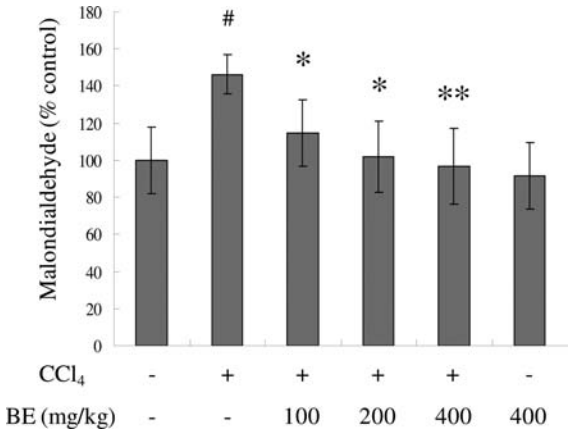

Figure 2. Effect of BE on the levels of lipid peroxidation in rat liver. Rats were treated with 1 g/kg CCl_4, CCl_4 plus 100 mg/kg BE, CCl_4 plus 200 mg/kg BE, CCl_4 plus 400 mg/kg BE, and 400 mg/kg BE, as described under Materials and Methods. Tissue homogenates were prepared from rat liver and tested for levels of lipid peroxidation. Data are presented as the mean ± SD of six rats. (#) $p < 0.01$ versus control; (*) $p < 0.05$ versus CCl_4; (**) $p < 0.01$ versus CCl_4.

Effects of BE on the Antioxidant Enzyme Activities in the Liver of CCl_4-Treated Rats. In living organisms, the production of free radicals is prevented by antioxidant enzymes such as SOD, catalase, GPx, and GR. SOD is an effective defense enzyme that catalyzes the dismutation of the superoxide anion ($\bullet O_2^-$) to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). Catalase is a ubiquitous antioxidant enzyme that is involved in the detoxification of H_2O_2. GPx and GR are important enzymes in the reduction of hydroperoxides to nontoxic products. GPx converts H_2O_2 to O_2 and H_2O using the glutathione (GSH) as the electron donor. GR promotes the NADPH-dependent reduction of oxidized glutathione (GSSG) by GPx to glutathione. Many studies have reported that free radicals induced by CCl_4 reduced the activity of antioxidant enzymes, such as SOD, catalase, GPx, and GR.[3,5,25,26] To evaluate the protective effect of BE on oxidative stress in CCl_4-treated rats, we therefore examined the activities of antioxidant enzymes. The activities of antioxidant enzymes SOD and CAT were reduced by approximately 30% in CCl_4-treated rats compared with the control group. However, preadministration of BE increased SOD and CAT activities in a dose-dependent manner compared to rats treated with CCl_4 alone (Figure 3A,B). GPx and GR activities in the liver were reduced in CCl_4-treated rats when compared with the control group. Treatment with various concentrations of BE

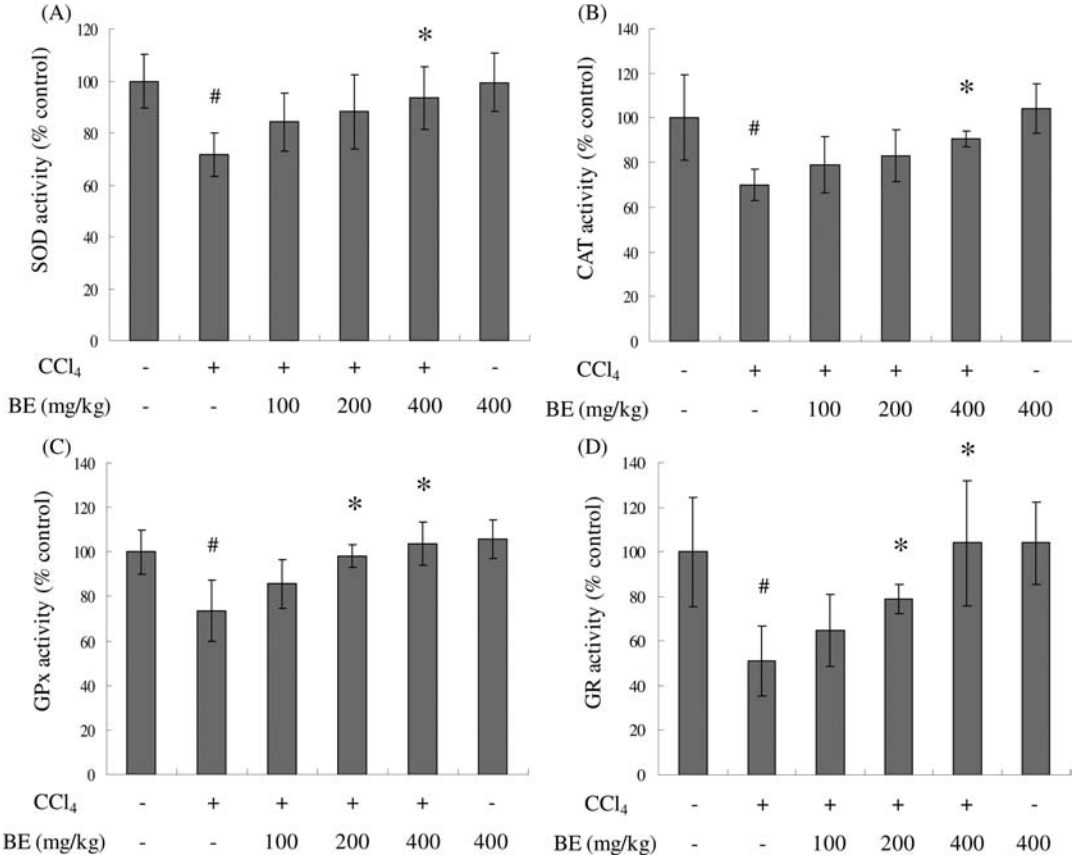

Figure 3. Effect of BE on the SOD, CAT, GPx, and GR activity in rat liver. Rats were treated with 1 g/kg CCl_4, CCl_4 plus 100 mg/kg BE, CCl_4 plus 200 mg/kg BE, CCl_4 plus 400 mg/kg BE, and 400 mg/kg BE, as described under Materials and Methods. Tissue homogenates were prepared from rat liver and tested for SOD activity (A), CAT activity (B), GPx activity (C), and GR activity (D). Data are presented as the mean ± SD of six rats. (#) $p < 0.05$ versus control; (*) $p < 0.05$ versus CCl_4.

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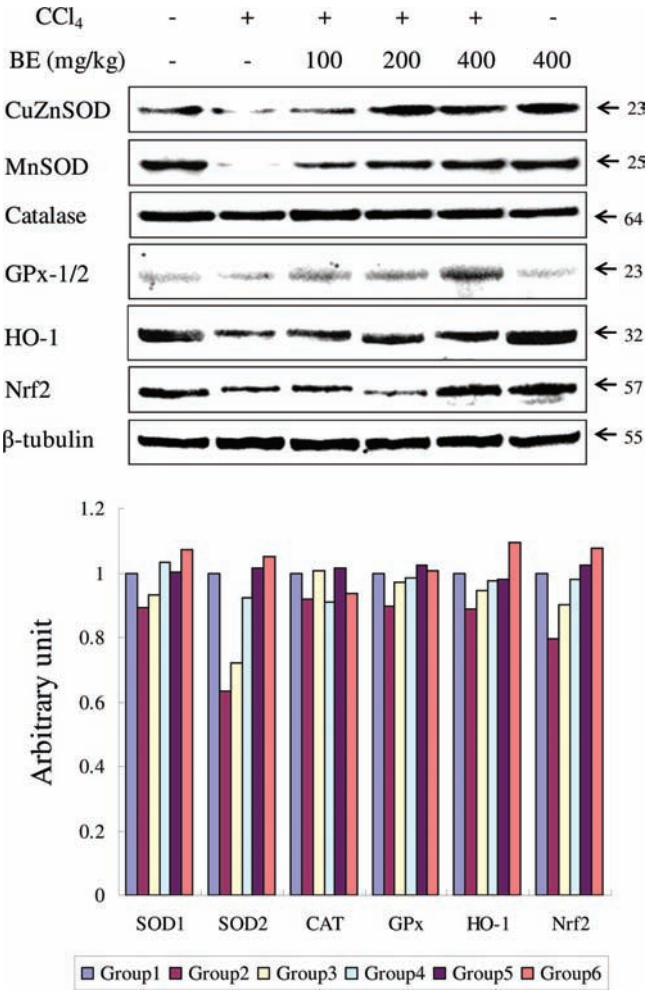

Figure 4. Effect of BE on protein levels of antioxidant enzymes and Nrf2 in rat liver. Fifty micrograms of protein was electrophoresed on a 12% polyacrylamide gel. After electrophoresis, the proteins were transferred to a nitrocellulose membrane and detected by Western blot analysis. β-Tubulin served as a control.

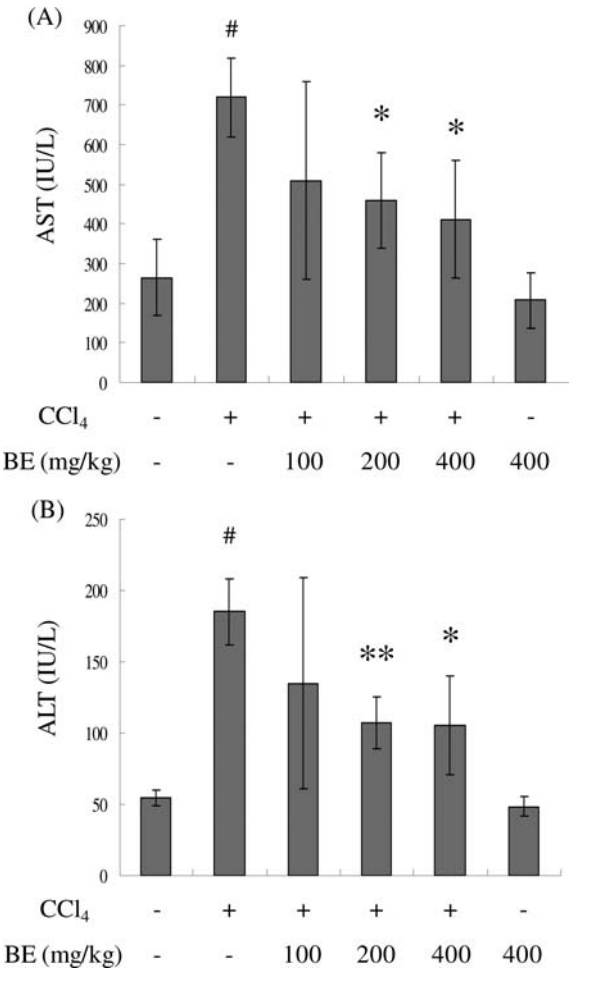

Figure 5. Effect of BE on the levels of serum AST and ALT in CCl_4-treated rats. Rats were treated with 1 g/kg CCl_4, CCl_4 plus 100 mg/kg BE, CCl_4 plus 200 mg/kg BE, CCl_4 plus 400 mg/kg BE, and 400 mg/kg BE, as described under Materials and Methods. Blood samples were prepared from rats and tested for AST and ALT levels. Data are presented as the mean ± SD of six rats. (#) $p < 0.001$ versus control; (*) $p < 0.05$ versus CCl_4; (**) $p < 0.01$ versus CCl_4.

caused a significant increase in GPx and GR activities when compared with the CCl_4-treated group (Figure 3C,D). There was no significant difference in activity between the control group and rats treated with BE alone. These results indicate that BE attenuated oxidative stress induced by CCl_4 through increased activity of antioxidant enzymes. These elevated enzyme activities are consistent with the results from the lipid peroxidation assay.

Effects of BE on the Expression of Nrf2 and Antioxidant Enzymes. Nrf2 plays an important role in the activation of antioxidant and detoxifying enzymes such as SOD, catalase, GPx, GR, glutamate cysteine ligase (GCL), NAD(P)H:quinine oxidoreductase 1 (NQO-1), and heme oxygenase 1 (HO-1) by regulating their transcription.[27,28] We thus measured the protein expression levels of Nrf2 and antioxidant enzymes. Nrf2 expression in the CCl_4-treated groups decreased significantly compared with the control group. The expression of antioxidant enzymes by CuZnSOD, MnSOD, GPx-1/2, and HO-1 also decreased, but not that of catalase. However, the administration of BE increased the expression levels of Nrf2, CuZnSOD, MnSOD, and GPx-1 in the CCl_4-treated rats in a dose-dependent manner. An increase of HO-1 expression was also observed in the BE-treated groups in a dose-dependent manner (Figure 4). Recently, it has been reported that the induction of Nrf2 and antioxidant enzymes such as CuZnSOD, MnSOD, CAT, GPx-1, and γ-GCS plays an important role in protection against oxidative stress in *tert*-butyl hydroperoxide-treated mice.[29] Sahin et al.[30] have also demonstrated that the administration of epigallocatechin-3-gallate increases antioxidant enzymes, such as CuZnSOD, MnSOD, CAT, GPx, and HO-1, through activation of Nrf2 in rats. Although CAT expression is not increased, our results correspond with previous studies.[29,30] Therefore, our results suggest that the antioxidant activity of BE may be related to the increase of protein expression of antioxidant enzymes through the activation of Nrf2.

Effects of BE on the AST and ALT Levels in CCl_4-Treated Rat Serum. The activities of serum AST and ALT are the most commonly used to test for liver damage. Liver damage by CCl_4 increases the permeability of the plasma membrane and releases AST and ALT, which are known markers of acute hepatic damage, from the liver into blood.[31,32] Accordingly, the levels of serum AST and ALT were estimated as an evaluation of liver injury. As shown in Figure 5, treatment of CCl_4 resulted in a

significant increase in the levels of AST and ALT compared with the control group. However, the administration of BE at three different concentrations attenuated the increased levels of AST and ALT enzymes by CCl_4. These data suggest that BE has a protective ability against acute hepatic damage by oxidative stress. The results correspond with the reduced oxidative liver injury measured by the lipid peroxidation assay.

Many berry fruits are rich in phenolic compounds such as phenolic acids, flavonoids, and anthocyanins and have been reported to have antioxidant, anticancer, and anti-inflammatory properties. Because of the biological properties associated with berry fruits, these fruits are consumed fresh or as processed products such as jams, jellies, syrups, and wines.[9,24] Therefore, our data indicate that blackberry consumption may contribute to human health.

In conclusion, our results showed that BE inhibited lipid peroxidation and increased the activity and the protein expression of antioxidant enzymes via the activation of Nrf2 in CCl_4-treated rats. In addition, BE significantly decreased AST and ALT levels in CCl_4-treated rats. Therefore, the results of this study suggest that blackberry extract may be useful as a potential agent against oxidative stress.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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