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Protective Effects of Bilberry (*Vaccinium myrtillus* L.) Extract on Restraint Stress-Induced Liver Damage in Mice

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Our experiments showed that 18 h restraint stress could induce serious liver damage, with an increase in plasma alanine aminotransferase (ALT) level (107.68 ± 3.19 U/L vs 18.08 ± 1.46 U/L). Meanwhile, we observed increased malondialdehyde (MDA) levels and lowered oxygen radical absorbance capacity (ORAC) values in plasma and liver of restraint mice compared with starved mice. Bilberry extract (containing 42.04% anthocyanins) was oral administrated to mice at 50, 100, and 200 mg/(kg·day) for five days, which remarkably decreased plasma ALT level to 17.23 ± 2.49 U/L at the dose of 200 mg/(kg·day) and thus alleviated stress-induced liver damage. In addition, bilberry extracts increased glutathione (GSH) and vitamin C levels and significantly decreased MDA and nitric oxide (NO) levels in the liver tissues. These results suggest that bilberry extract plays an important role in protecting against restraint stress-induced liver damage by both scavenging free radicals activity and lipid peroxidation inhibitory effect. This study showed the beneficial health effects of bilberry extract through its antioxidative action.

KEYWORDS: Bilberry (*Vaccinium myrtillus* L.) extract; anthocyanins; restraint stress; oxidative stress; liver damage

INTRODUCTION

It has been widely reported that long-term exposure to stress induces free radical reactions leading to deleterious modifications in membranes, proteins, enzymes, and DNA (*1*). Stress suppresses the immune system, affects the secretion of hormones, increases lipid peroxidation, and decreases the amount of endogenous antioxidative substances such as GSH and vitamin E (*2, 3*). Diminished antioxidant protection and increased oxidative stress may be correlated with lifestyle-related diseases such as arteriosclerosis (*4*) and Alzheimer disease (*5*).

Nutritionists have sought to understand the body's oxidation processes and to prevent damages caused by rogue oxygen molecules (*6*). Several studies have indicated that antioxidants could quench free radicals or suppress the generation of free radicals by interrupting oxidation chain reactions (*7*). Antioxidants that trap free radicals and lipid peroxides may delay the onset of lipid peroxidation, inhibiting the production of free radicals and suppressing damages induced by enzymes that can degrade connective tissues (*8*).

Bilberry (*Vaccinium myrtillus* L.), a native of Europe and North America, is a low-growing shrub of the Eriacaceae family. It has been found to play a protective role in human health maintenance due to its various biomedical activities, such as cardiovascular disorders, advancing age-induced oxidative stress, inflammatory responses, diverse degenerative diseases, and trigger genetic signaling in promoting human health and disease prevention. Bilberry extract is available in the market as a pharmaceutical preparation for the treatment of both ophthalmologic diseases and blood vessel disorders. A significant increase in plasma antioxidant capacity was observed following consumption of anthocyanins juice (*9*). Its potent biological properties can be correlated to the high content of anthocyanins pigment. Recently, bilberry extract has been reported to possess antioxidative activity and cytoprotective effect against oxidative damage in various models in vitro (*10, 11*). In this study, the effect of anthocyanins on the alanine aminotransferase (ALT) level and antioxidative parameters in mice were investigated using a commercially available standardized anthocyanin extract

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(containing 42.04% anthocyanins, Indena S.P.A, Milan, Italy) from bilberry.

Little is known about the effects of bilberry extract on restraint stress. Therefore, we evaluated the antistress effects of bilberry extract by examining oxygen radical absorbance capacity (ORAC), glutathione (GSH), and vitamin C levels in mice exposed to restraint stress. Restraint, which has an extensive history, has been widely used for the studies of animal physiology, pathology, and pharmacology and has proven to be very useful for examination of stress-related disorders, as well as for studying drug effects upon these disorders (*12*). Restraint stress promotes lipid peroxidation in liver tissue (*13*), leading to oxidative damage by changing the balance between oxidant and antioxidant factors. We believe bilberry extract might exert a protective effect on liver damage caused by oxidative stress.

MATERIALS AND METHODS

Materials and Chemicals. Bilberry extract was purchased from Indena S.P.A (Milan, Italy) (batch no. 28870/M). Authentic standards of malvidin-3-*O*-galactose, malvidin-3-*O*-glucose, cyanidin-3-*O*-galactose, and cyanidin-3-*O*-glucose were obtained from Extrasynthese S.A (Genay, France). The anthocyanins in bilberry were analyzed by ESI-MS/RP-HPLC at 535 nm. The analysis was performed with a Waters RP-18 column (4.6 mm × 250 mm) with the following mobile phase: (A) water/formic acid (90:10, v/v) and (B) methanol/acetonitrile/water/formic acid (22.5:22.5:40:10, v/v/v/v). The solvent gradient was held at 9% B in the initial 45 min and then increased from 9 to 35% B in the following 45 min. ESI-MS was in positive ion mode. The concentrations of 15 anthocyanins in bilberry were shown in **Table 1** (*14*).

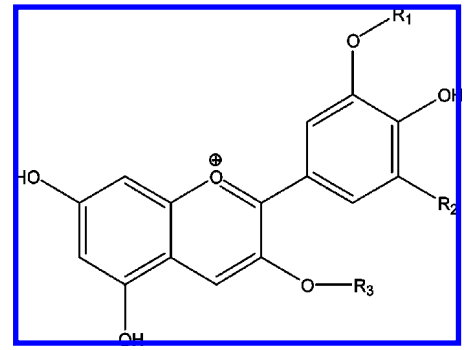
Vitamin C and GSH were purchased from Sigma Chemical Co. Methanol was obtained from Fisher Scientific. ALT kit, XOD kit, MDA kit, and albumin quantitation kit were obtained from Nanjing Jiancheng Bioengineering Institute, China. Sodium 1-octanesulfonate (SOS) was purchased from Kasei Kogyo Co. Ltd. (Tokyo, Japan). 2,2-Azobis (2-amidinopropane) dihydrochloride (AAPH), sodium fluorescein (FL), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carcoxylic acid (Trolox, a water-soluble vitamin E analogue) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Animals and Treatments. 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 with a 12-h light−dark cycle (lights on from 06:00 to 18:00) and were provided with standard laboratory diet and tap water. The animals were allowed to acclimatize to the environment for 1 week before the experiment.

In the present study, mice were randomly divided into six groups with 10 animals each. In the restraint stress experiment, each mouse was confined to an oval metal restraint cage for 18 h before the assay (**Figure 1**). As the mice could not be fed by any food for 18 h, a starved control group in which mice were starved for 18 h without restraint stress was set up. Starved control and restraint stress control were fed water once daily for 5 days at a dose of 0.1 mL/10 g body weight. Bilberry extract was dissolved in water before use, and the solution was orally administered to animals at 0.1 mL/10 g body weight for 5 days at dosages of 50, 100, and 200 mg/(kg·day) before exposure to restraint stress (**Figure 1**). Another group of mice were given oral administration of vitamin C once daily for 5 days at a dose of 200 mg/(kg·day). All animals were sacrificed after restraint stress. The care and treatment of the animals conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication no. 85-23, revised 1985), and the experiment was in accordance with animal ethics standards.

Plasma, Tissue Samples Collection, and Protein Level Measurement. Under ether anesthesia, blood was collected in test tubes with heparin sodium (50 U/mL blood) following cardiac puncture, and the liver was quickly removed. Blood samples were centrifuged at 5 000 rpm for 10 min at 4 °C by refrigerated centrifuge (Sigma Co., Germany) to obtain the plasma. Liver samples were homogenized in chilled 0.01 M PBS (pH 7.4) using an ULTRA-TURRAX T8 homogenizer (GmbH Co., Germany) and centrifuged at 10 000 rpm for 10 min at 4 °C. A 2% liver homogenate was used to determine the protein concentration using a Coomassie brilliant blue kit with bovine serum albumin as the standard.

Table 1. Structure and Concentration of 15 Anthocyanins in Bilberry Extract[a]



	structure				
ingredient[b]	R1	R2	R3	+MS	conc (mg/g)
delphinidin-3-*O*-gal	H	OH	galactose	303, 465	49.07
delphinidin-3-*O*-glu	H	OH	glucose	303, 465	54.21
cyanidin-3-*O*-gal*	H	H	galactose	287, 449	40.03
delphinidin-3-*O*-ara	H	OH	arabinose	303, 435	49.06
cyanidin-3-*O*-glu*	H	H	glucose	287, 449	41.81
cyanidin-3-*O*-ara	H	H	arabinose	287, 419	32.18
petunidin-3-*O*-gal	H	CH_3O	galactose	317, 479	17.92
petunidin-3-*O*-glu	H	CH_3O	glucose	317, 479	38.49
peonidin-3-*O*-gal	CH_3	H	galactose	301, 463	4.34
petunidin-3-*O*-ara	H	CH_3O	arabinose	317, 449	13.44
peonidin-3-*O*-glu	CH_3	H	glucose	301, 463	15.90
malvidin-3-*O*-gal *	CH_3	CH_3O	galactose	331, 493	13.23
peonidin-3-*O*-ara	CH_3	H	arabinose	N.D.[c]	1.75
malvidin-3-*O*-glu *	CH_3	CH_3O	glucose	331, 493	40.15
malvidin-3-*O*-ara	CH_3	CH_3O	arabinose	331, 463	8.75
total anthocyanins					420.33

[a] The extract was analyzed by ESI-MS/RP-HPLC (*15*). [b] Identities of cyanidin-3-*O*-gal, cyanidin-3-*O*-glu, malvidin-3-*O*-gal, and malvidin-3-*O*-glu (marked by *) were confirmed by comparison with authentic standards; other compounds were assigned according to ESI-MS data found in the literature (*15*). [c] N.D. = not determined.

Measurement of Plasma ALT Level. ALT levels in the plasma were measured by Reitman-Frankel method using a commercial kit. ALT reacted with alanine and α-ketoglutaric acid to produce pyroracemic acid, In basylous medium and in the presence of dinitro-phenylhydrazine (DPNH), brown color was observed and measured at 492 nm using a MK_3 microplate reader (Labsystems Co., Finland).

Measurement of GSH and Vitamin C Levels in Liver. The concentration of GSH in the liver was determined by high-performance liquid chromatography (HPLC) (*16*): SC-5 ODS column (Eicom Co., 4.6 mm × 150 mm, 5 μm); mobile phase, 99% phosphate buffer (pH 2.5)−1% methanol containing 100 mg/L SOS and 5 mg/L EDTA; flow rate, 0.5 mL/min; and an electrochemical detection system (ECD-300, Eicom Co.) operated at room temperature.

The measurement of the vitamin C level was performed as the method described by Wu (*17*). The following chromatographic conditions were applied: a COSMOSIL ODS column (i.d. 4.6 mm × 150 mm) at room temperature, 99 mM potassium phosphate buffer (pH 3.0) consisting of 1% methanol at a flow rate of 1 mL/min, and UV detection at 245 nm.

For GSH and vitamin C analysis, 10% liver homogenate were deproteinized by 3% perchloric acid (PCA) and then centrifuged at 15 000 rpm for 15 min at 4 °C. The supernatant were injected after being filtered through a 0.45 μm filter disk.

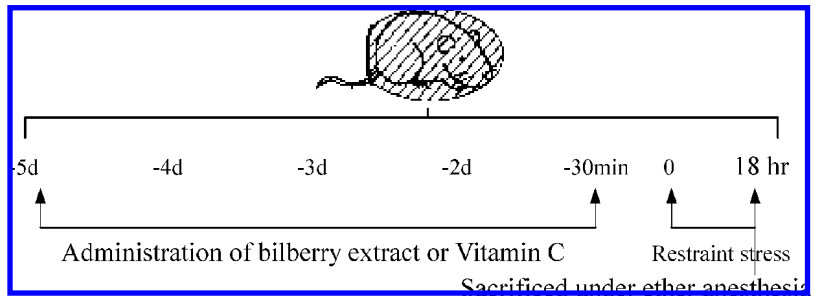


Figure 1. Experimental schedule for mice exposed to restraint stress. KM mice were fixed in the restraint cage for 18 h before the measurement of biochemical parameters.

Measurement of XOD and NO Levels in Liver. XOD levels in the liver were determined using a commercial XOD kit. XOD catalyzed the oxidation of hypoxanthine to xanthine to produce superoxide radical, which eventually results in pink adduct detected at 530 nm with a MK_3 microplate reader (Labsystems Co., Finland).

NO levels were determined by the Griess method (*18*). 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 20min, the purple-azo-dye product was detected at 540 nm with a MK_3 microplate reader (Labsystems Co., Finland).

Measurement of MDA Levels in Plasma and Liver. MDA levels in plasma and liver were measured with a commercial MDA kit. In acidic medium, MDA reacted with thiobarbituric acid (TBA) upon boiling, and the resultant MDA−TBA adducts were pink in color and measured by a MK_3 microplate reader (Labsystems Co., Finland) at 532 nm.

Measurement of ORAC in Plasma and Liver. Plasma samples obtained above were treated with 6% PCA. It was centrifuged at 15 000 rpm for 15 min at 4 °C; then, the supernatant was removed as the nonprotein plasma fraction and appropriately diluted in phosphate buffer (pH 7.4) for ORAC analysis.

For liver samples analysis, 2% liver homogenate was deproteinized by adding 3% PCA (1:1) and centrifuging at 15 000 rpm for 15 min at 4 °C. The supernatant was subsequently stored at −80 °C for further assay.

Automated ORAC assay was carried out on a Labsystems Fluoroskan Ascent plate reader (Helsinki, Finland) with fluorescent filters (Infinite F200, excitation wavelength, 485 nm; emission wavelength, 527 nm) as previously described (*19*). AAPH was used as a radical generator, and the reaction was initiated with fluorescein; trolox was used as a standard. Final results were calculated based on the difference in the area under the fluorescein decay curve between the AAPH control and each sample.

Statistical Analysis. The data were presented as mean ± SE. Statistical analysis of the data was performed using the SPSS 13.0 statistical package. 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 posthoc test for pairwise multiple comparisons. Differences were considered as statistically significant at $p < 0.05$ level.

RESULTS

Effects of Bilberry Extract on Plasma ALT Levels. The plasma ALT level in starved mice was 18.08 ± 1.46 U/L, while the one in 18 h stressed mice was 107.68 ± 3.19 U/L; stress significantly increased the plasma ALT level ($p < 0.001$). Bilberry extract at 200 mg/(kg·day) decreased the plasma ALT levels to 17.23 ± 2.49 U/L, which have been recovered to that of the starved control (**Table 2**).

Effects of Bilberry Extract on GSH and Vitamin C Levels in Liver Tissue. The liver GSH level in restraint stress mice significantly lowered compared with the starved mice ($p < 0.001$). Bilberry extract at 200, 100, and 50 mg/(kg·day) as well as 200 mg/(kg·day) vitamin C significantly increased the liver tissue GSH levels compared with the stressed mice.

Table 2. Effects of Bilberry Extract on Plasma Biomarkers in Mice Treated with 18 hr Restraint Stress

treatment[a]	ALT (U/L)	MDA (nmol/mL)	ORAC (μM Trolox equiv)
starved control	18.08 ± 1.46	11.52 ± 1.17	44.00 ± 2.77
restraint control	107.68 ± 3.19[b]	20.08 ± 0.82[b]	36.83 ± 1.52[c]
restraint + Vc (200 mg/(kg·day))	42.69 ± 2.09[d]	16.48 ± 0.52[e]	41.90 ± 2.29
restraint + BE (50 mg/(kg·day))	55.82 ± 1.51[d]	18.12 ± 1.16	40.06 ± 2.11
restraint + BE (100 mg/(kg·day))	37.38 ± 1.55[d]	14.96 ± 0.64[d]	44.02 ± 1.01[e]
restraint + BE (200 mg/(kg·day))	17.23 ± 2.49[d]	14.16 ± 0.66[d]	46.61 ± 0.94[d]

[a] BE = bilberry extract. Seven-week-old male KM mice were confined in restraint cages for 18 h before measurement of plasma biomarkers. The results represent mean ± SE obtained from 10 animals in each group. [b] Significantly different from starved control mice at $p < 0.001$. [c] Significantly different from starved control mice at $p < 0.05$. [d] Significantly different from restraint control mice at $p < 0.001$. [e] Significantly different from restraint control mice at $p < 0.05$ (One-way ANOVA followed by Dunnett's test).

Stress also lowered the liver vitamin C level (173.41 ± 24.0 μg/g tissue) compared with the starved mice (399.74 ± 22.0 μg/g tissue). Compared with the control group, 200 and 100 mg/(kg·day) of bilberry extract significantly increased liver vitamin C levels to 450.87 ± 19.8 and 346.67 ± 28.4 μg/g tissue ($p < 0.001$). Furthermore, the level of liver vitamin C in the 200 mg/(kg·day) vitamin C group also significantly increased ($p < 0.001$) (**Table 3**).

Effects of Bilberry Extract on XOD and NO Levels in Liver Tissue. Table 3 showed the increase of the liver XOD level after 18 h restraint stress. The ANOVA analysis revealed that five-day oral intake of bilberry extract significantly reduced XOD level in treated mice.

In addition, stress increased the liver NO level (19.65 ± 0.80 μmol/mL) compared with the starved mice (15.98 ± 0.89 μmol/mL) (**Table 3**). Compared with the stress control group, 200 and 100 mg/(kg·day) of bilberry extract significantly decreased NO levels, respectively. Furthermore, the level of liver NO in the 200 mg/(kg·day) vitamin C group also significantly increased compared with the stressed mice (15.76 ± 0.76 μmol/mL, $p < 0.001$).

Effects of Bilberry Extract on MDA Level in Liver and Plasma. The liver MDA level in stressed mice was significantly higher than that in the starved mice (2.22 ± 0.20 nmol/mg of protein versus 1.53 ± 0.16 nmol/mg of protein, $p < 0.05$) (**Table 3**). At 200 mg/(kg·day), bilberry extract could suppress and reverse the liver MDA to a level comparable to the starved control. Vitamin C at 200 mg/(kg·day) also significantly decreased the liver MDA level (1.67 ± 0.18 nmol/mg of protein) compared with the stressed mice ($p < 0.05$).

Table 3. Effects of Bilberry Extract on Liver Biomarkers in Mice Treated with 18 hr Restraint Stress

treatment[a]	GSH (μg/g tissue)	vitamin C (μg/g tissue)	XOD (U/g protein)	NO (μmol/mL)	MDA (nmol/mg protein)	ORAC (μMTrolox equiv)
starved control	1415.2 ± 167.4	399.74 ± 22.0	3.00 ± 0.19	15.98 ± 0.89	1.53 ± 0.16	22.86 ± 2.70
restraint control	495.4 ± 34.4[b]	173.41 ± 24.0[b]	4.58 ± 0.32[b]	19.65 ± 0.80[b]	2.22 ± 0.20[c]	14.07 ± 2.97[b]
restraint + Vc (200 mg/(kg·day))	1236 ± 102.2[d]	480.92 ± 24.4[d]	4.10 ± 0.42	15.76 ± 0.76[d]	1.67 ± 0.18[f]	22.39 ± 3.66[e]
restraint + BE (50 mg/(kg·day))	820.9 ± 54.8[f]	233.64 ± 30.6	3.19 ± 0.85[e]	18.08 ± 0.52	2.01 ± 0.15	28.47 ± 2.15[e]
restraint + BE (100 mg/(kg·day))	1087.9 ± 71.8[d]	346.67 ± 28.4[d]	2.65 ± 0.94[d]	16.82 ± 0.58[e]	1.81 ± 0.24	23.76 ± 3.16[e]
restraint + BE (200 mg/(kg·day))	1367.3 ± 60.6[d]	450.87 ± 19.8[d]	2.10 ± 0.52[d]	14.63 ± 0.65[d]	1.48 ± 0.13[e]	20.87 ± 2.08[e]

[a] BE = bilberry extract. Seven-week-old male KM mice were confined in restraint cages for 18 h before measurement of liver biomarkers. The results represent mean ± SE obtained from 10 animals in each group. Significantly different from starved control mice at [b] Significantly different from starved control mice at $p < 0.001$. [c] Significantly different from starved control mice at $p < 0.05$. [d] Significantly different from restraint control mice at $p < 0.001$. [e] Significantly different from restraint control mice at $p < 0.01$. [f] Significantly different from restraint control mice at $p < 0.05$ (One-way ANOVA followed by Dunnett's test).

Similarly, stress increased the plasma MDA level (20.08 ± 0.82 nmol/mL) compared with the starved mice (11.52 ± 1.17 nmol/mL) (**Table 2**). Compared with the stress control group, 200 and 100 mg/(kg·day) of bilberry extract significantly decreased the plasma MDA levels, and the level of plasma MDA in the 200 mg/(kg·day) vitamin C group was also significantly lower than that in the stressed mice group ($p < 0.05$).

Effects of Bilberry Extract on ORAC Levels in Liver and Plasma. The liver ORAC level in starved mice was 1.62-fold higher as compared with 18 h stressed mice. Bilberry extract at 200, 100, and 50 mg/(kg·day) suppressed the decrease of the liver ORAC level in stressed mice to 20.87 ± 2.08, 23.76 ± 3.16, and 28.47 ± 2.15 μM Trolox equiv, respectively (**Table 3**).

Stress also decreased the plasma ORAC level (36.83 ± 1.52 μM Trolox equiv) compared with the starved mice (44.00 ± 2.77 μM Trolox equiv) (**Table 2**). Compared with the stress control group, 200 and 100 mg/(kg·day) of bilberry extract significantly increased ORAC levels.

DISCUSSION

In recent years, numerous studies have shown that bilberry extract scavenged superoxide anion and hydroxyl radicals and inhibited liver lipid peroxidation in rat and mice (*20*). Increase in plasma ALT activity, a marker of liver damage, was observed in restrained mice (*21*). Our results showed that mice pretreated with bilberry extract demonstrated decreased plasma ALT levels, suggesting that bilberry extract could attenuate restraint stress-induced liver damage through its antistress effects. We also observed the effects of restraint as a physiological stressor in the ORAC activity of plasma and liver in mice. Mice subjected to restraint for 18 h showed an approximately 38% decrease in their liver tissue ORAC level showing that restraint stress accelerated the formation of ROS, which was reported previously by our group (*22*). The GSH and vitamin C levels were also reduced significantly. These results suggested that restraint stress plays a role in increasing oxidative stress as demonstrated by Mugbil and Banu (*23*). In general, total antioxidant capacity of the liver tissue is regulated through a homeostatic mechanism in part and is due to a variety of compounds including GSH, vitamins C and E, and possibly other endogenous components. GSH is a very important antioxidant found in tissues. Early studies have given clear evidence that decreased liver blood flow induced by stress may attenuate GSH synthesis from circulatory sources via the γ-glutamyl cycle (*24*), as well as causing disturbances in GSH homeostasis, such as decreasing its plasma concentration, degrading its cellular redox status, and interfering with GSH transport (*25*). Although endogenous GSH content is not sufficient to increase oxidative status, it is beneficial for the cell to increase its GSH level so that they can quench reactive oxygen species in the restraint treated mice.

We also observed that bilberry extract significantly ameliorated decreased the ORAC level in plasma and liver of stressed mice. The ORAC level reflects the antioxidative capacity of water-soluble low molecular antioxidants such as GSH and vitamin C (*3, 26*). Our results showed that bilberry extract attenuated oxidative stress by changing the oxidative status and improving antioxidative processes in mice subjected to stress.

Restraint stress also lowered the liver vitamin C level compared with the starved mice. Humans could not synthesize vitamin C because they lack the functional gene (*Gulo*) coding for a key synthetic enzyme, L-gulono-γ-lactone oxidase, whereas mice having this functional gene and can synthesize vitamin C on their own; as a consequence, mouse tissues generally have high levels of vitamin C. But, our previous study found vitamin C in mice is prone to be reduced by restraint stress (*22*). Ingestion of bilberry extract by restraint stressed mice led to increased vitamin C levels probably by suppressing the oxidative degradation of vitamin C. Enhanced liver lipid peroxidation was observed in restrained mice, suggesting that cells deficient in thiol groups undergo MDA accumulation. Numerous studies have indicated that immobilization stress induced an increase in plasma MDA level. We found that the increase of MDA was accompanied by significant change of XOD level. XOD is the key enzyme for producing ROS under pathology condition, which catalyzes the oxidation of hypoxanthine to xanthine and produces free radicals, causing cellular structure damage and functional diabolisms. In the present study, significantly elevated NO level was observed in liver tissue of the restraint-stressed mice. Excess NO will react with oxygen free radical to produce the cytotoxic radical $ONOO^-$ that could damage cellular functions. Therefore, the high plasma ALT level after 18 h restraint stress may be explained by liver's high lipid peroxidation and the accumulation of ROS, as well as the decreased GSH and vitamin C levels. Therefore, exposure to restraint stress may increase the oxidative damage of protein structures, especially in the liver tissue.

Bilberry extract is widely known to be an antioxidant. However, less attention has been given to its effect on stress. In the present study, the protection against oxidative damage might be linked to the antioxidative properties of the anthocyanins in bilberry extract. Our analysis result showed that bilberry extract contained 420.33 mg/g anthocyanins and has strong activity in scavenging the free radicals generated by AAPH and protecting kidney from damage induced by $KBrO_3$ (*14*). In Valentova's study, bilberry extract containing 25% anthocyanins displayed cytoprotective effect against oxidative damage of rat hepatocytes induced by *tert*-butylhydroperoxide and allyl alcohol (*11*).

The results obtained from our study indicated that oxidative stress plays an important role in restraint-induced liver damage

and confirmed that bilberry extract acts as a potent scavenger of free radicals to prevent the toxic effects of restraint stress in the liver. The present work encourages us to further investigate the pharmacodynamics of bilberry extract for its effect on lifestyle-related diseases and general health.

ABBREVIATIONS USED

ALT, alanine aminotransferase; MDA, malondialdehyde; ORAC, oxygen radical absorbance capacity; GSH, glutathione; SOS, 1-octanesulfonate; AAPH, 2-azobis (2-amidinopropane) dihydrochloride.

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