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The Protective Effect and Action Mechanism of *Vaccinium myrtillus* L. on Gastric Ulcer in Mice

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***Vaccinium myrtillus* L. anthocyanoside (VMA) is used as a folk medicine to treat diseases related to gastric ulcers in northern Europe. However, the effects of VMA and its detailed mechanism on gastric ulcer have not been investigated sufficiently. Therefore, the aim of the present study was to investigate the protective effects of VMA on gastric mucosal damage in a murine gastric ulcer model. First the effects of VMA on ethanol-induced gastric ulcers in mice were investigated. Then, the levels of lipid peroxide in murine stomach homogenates were measured to investigate the antioxidative effects of VMA. In addition, the free radical scavenging activity of VMA and its main anthocyanidins were evaluated by electron spin resonance measurement. Oral administration of VMA (10, 30 and 100 mg/kg) significantly protected gastric mucosa against HCl/ethanol-induced gastric ulcers. Furthermore, VMA inhibited lipid peroxide levels in a concentration-dependent manner and showed high scavenging activity against the superoxide anion radical ($\cdot O_2^-$) and the hydroxyl radical (·OH). Anthocyanidins also showed scavenging activity against the $\cdot O_2^-$, while only delphinidin showed high scavenging activity against the ·OH. These findings indicate that the protective effects of VMA on HCl/ethanol-induced gastric mucosal injury may be partially due to the antiperoxidative effects of anthocyanidins. Copyright © 2011 John Wiley & Sons, Ltd.**

Keywords: gastric acid; *Vaccinium myrtillus* L.; anthocyanidins.

INTRODUCTION

Gastric ulcer is one of the most widespread diseases in the world and occurs with stress, nonsteroidal antiinflammatory drugs, surfeit, *Helicobacter pylori* infection and alcohol ingestion. Its pathogenesis involves the generation of oxygen-derived free radicals such as the superoxide anion radical and the hydroxyl radical, the infiltration of neutrophils and the activation of neutrophils. Furthermore, it has been shown that lipid peroxidation plays an important role in the pathogenesis of gastric mucosal lesions (Del Soldato *et al*., 1986; Terano *et al*., 1989; Das and Banerjee, 1993).

Ethanol is a well-known agent that has been shown to induce damage to the gastric mucosa in animal and clinical studies. Oral administration of ethanol decreases the gastric mucosal barrier, liberates histamine and elicits inflammation in gastric mucosal cells. Ethanol also causes marked mucosal hyperemia, necrosis, edema and mucosal or submucosal hemorrhage (Lulu and Dragstedt, 1970; Eastwood and Kirchner, 1974; Tarnawski *et al*., 1981; Szabo, 1987). The formation of lesions may be mediated by oxygen-derived free radicals (Del Maestro *et al*., 1981; Pihan *et al*., 1987; Szelenyi and Brune, 1988; Terano *et al*., 1989; Shaw *et al*., 1990). Antioxidant agents, such as vitamin E (Tariq, 1988; Jaarin *et al*., 1999), catechin (Hamaishi *et al*., 2006), quercetin (Ömer *et al*., 2004) and astaxanthin (Jeong-Hwan Kim *et al*., 2005) are known to inhibit the products of lipid peroxidation and subsequently, ethanol-induced gastric ulcers in animal models.

Vaccinium myrtillus L. (bilberry), a member of the Ericaceous family, grows in the mountains and forests of northern Europe and North America. Bilberry extracts containing 15 different anthocyanins have been shown to possess potent antioxidant properties and to improve blood flow (Colantuoni *et al*., 1991). Additionally, *Vaccinium myrtillus* L. anthocyanoside (VMA) has been reported to inhibit various gastric ulcers (restraint plus cold stress, phenylbutazone, indomethacin, reserpine, histamine, cysteamine, acetic acid and ethanol-induced ulcer models) (Magistretti *et al*., 1988), and is prescribed as a therapeutic drug for gastric ulcers in Italy. However, the mechanism of its protective effect against gastric ulcers has not been studied sufficiently, and there are no reports on the effect of VMA on murine gastric ulcers. The present study first investigated the effect of VMA on an HCl/ethanol-induced gastric ulcer model in mice. Second, the effects of VMA against auto-oxidation of lipid peroxidation were assayed in murine stomach homogenates. Finally, the radical-scavenging activity of VMA and its main anthocyanidins, such as delphinidin, cyanidin and malvidin, against the superoxide anion radical and the hydroxyl radical were measured using electron spin resonance (ESR) spectroscopy.

MATERIALS AND METHODS

Animals. Male ddY mice (6 weeks old; weighing 29–31 g; Japan SLC Ltd, Shizuoka, Japan) were used in the *in vivo* experiment. The animals were housed in an air-conditioned room at 24 ± 2 °C under a 12-h light/dark

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cycle (lights on 0800–2000). Each animal was used for one experiment only. All procedures relating to animal care and treatment conformed to the Animal Care Guidelines of the Animal Experiment Committee of Gifu Pharmaceutical University.

Materials. *Vaccinium myrtillus* L. anthocyanoside (VMA) (containing more than 25% anthocyanidins) was kindly donated by Wakasa Seikatsu Co., Ltd (Kyoto, Japan). The compositions of anthocyanidins in VMA were about 9.0%, 5.4% and 3.4% of delphinidin, cyanidin, and malvidin, respectively. Delphinidin, cyanidin and malvidin chloride were purchased from Extrasynthese (Genay Cedex, France). Sucralfate and trolox were purchased from Sigma-Aldrich (St Louis, MO, USA). α-Tocopherol was purchased from Wako Pure Chemical Industries, Ltd, (Osaka, Japan).

HCl/ethanol-induced gastric ulcer. After 24 h of fasting, the mice were divided randomly into seven groups. One group was given 0.1 mL/10 g of vehicle (0.5% carboxymethylcellulose solution) (Wako), four groups were treated with VMA (3, 10, 30 and 100 mg/kg, p.o.) and the other groups were treated with sucralfate (100 mg/kg, p.o.) or α-tocopherol (100 mg/kg, p.o.), as positive controls. The mice were administered 150 mM HCl/ethanol (0.1 mL/20 g body weight) after 1 h of sample treatment. The mice were killed by cervical dislocation after 1 h of ethanol treatment and their stomachs were removed and fixed with 1 mL of 1% formalin for 30 min. Then, the stomachs were opened along the greater curvature and gently rinsed with saline. The gastric ulcers were evaluated according to the area of gastric lesion by using specific image analysis software (Angiogenesis Image Analyzer, Kurabo, Osaka, Japan).

Histological analysis. Histological evaluation was performed on the glandular stomach of mice. The tissue samples were preserved in 10% buffered formalin and processed for routine paraffin block preparation. Sections about 5 μm in thickness were cut and stained with hematoxylin and eosin. Mucosal injury evaluation was performed under light microscopy, and the histopathological alterations were assessed by comparing lesions.

Lipid peroxidation in murine stomach tissue homogenates. The supernatant fraction of stomach tissue homogenates in normal mice was prepared. Stomach tissues were homogenized in a homogenizer (Physcotron NS-51, Microtec Co., Ltd, Chiba, Japan) in 30% w/v of ice-cold potassium chloride solution. Then, 945 μL portions of the diluted homogenate were added to 5 μL of VMA solution (concentration: 0.1, 1, 10 and 100 μg/mL) and 50 μL of 1 mM $FeSO_4 \cdot 7H_2O$, and incubated at 37 °C for 60 min. The reaction was stopped by adding 200 μL of 35% $HClO_4$, followed by centrifugation at 3000 rpm, 10 °C for 10 min. The supernatant (500 μL) was heated with 100 μL of 8.1% sodium dodecyl sulfate (SDS) solution and 500 μL of 20% acetic acid buffer (pH 3.6) containing 0.8% thiobarbituric acid (TBA) solution at 100 °C for 60 min. After the mixture was cooled on ice, 1 mL of butyl alcohol and pyridine (15:1) was added, and the sample was shaken gently for 5 min. After centrifugation at 4000 rpm for 10 min, the butyl alcohol–pyridine phase containing the thiobarbituric acid reactive substance (TBARS) was separated and its absorbance was measured at 532 nm.

Electron spin resonance (ESR) measurement. To measure the O_2^- and OH scavenging activity, an ESR spectrometer (JES-FA200, Jeol, Tokyo, Japan) equipped with manganese oxide (MnO) as an internal standard was used. Reactive oxygen species (ROS), OH and $\cdot O_2^-$ were analysed as the spin adducts of 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), DMPO-OH and DMPO-OOH, respectively. The following ESR settings were used: magnetic field, 335.6 ± 5; power, 4 (for DMPO-OOH) or 8 mT (for DMPO-OH); modulation frequency, 9.4 GHz; modulation amplitude, 2.0 × 0.1 mT; response time, 0.3 s; amplitude, 5 × 100; sweep width, 1.5 × 10 mT; and sweep time, 4.0 min. All measurements were performed at 25 °C (Rosen and Rauckman, 1984).

Superoxide radical scavenging activity. Superoxide anion radicals were generated by reaction of the hypoxanthine–xanthine oxidase system. Samples (VMA, delphinidin, cyanidin and malvidin) and the positive control (trolox) were dissolved in 0.1 M phosphate buffer solution (pH 7.4) containing 1% dimethyl sulfoxide (DMSO); hypoxanthine was dissolved in distilled water. Sample solution (50 μL), 5 mM hypoxanthine (50 μL), 230 mM DMPO (50 μL) and xanthine oxidase (0.4 units/mL) (50 μL) were mixed in a test tube, and the mixture was quickly transferred to a quartz flat cell (capacity 200 μL). Exactly 60 s after the addition of xanthine oxidase, the ESR spectra of the DMPO-OOH spin adducts were recorded (Noda *et al*., 1997, 1999).

Hydroxyl radical scavenging activity. The samples were dissolved in 0.1 M phosphate buffer solution (pH 7.4) containing 1% ethanol; $FeSO_4$ was dissolved in distilled water. Sample solution (50 μL), 18 mM DMPO (50 μL), 0.2 mM H_2O_2 (50 μL) and 0.02 mM $FeSO_4$ (50 μL) were mixed in a test tube, and the mixture was transferred quickly to a flat cell. Exactly 60 s after the addition of $FeSO_4$, the ESR spectra of the DMPO-OH spin adducts were recorded (Noda *et al*., 1997).

Statistical analysis. Data are presented as mean ± SEM. Statistical comparisons were made using a one-way analysis of variance (ANOVA) followed by a Student's *t*-test or Dunnett's multiple comparison test. A value of $p < 0.05$ was considered to indicate statistical significance.

RESULTS

HCl/ethanol-induced gastric ulcer in mice

To investigate the effects of VMA against HCl/ethanol-induced gastric ulcer, the mice were treated with VMA at 3, 10, 30 and 100 mg/kg, sucralfate at 100 mg/kg and α-tocopherol at 100 mg/kg. Multiple erosions in the glandular stomach were observed in the murine stomachs pretreated with vehicle 1 h after HCl/ethanol administration (Fig. 1B). Pretreatment with VMA (10, 30 and 100 mg/kg, p.o.) at 1 h before the HCl/

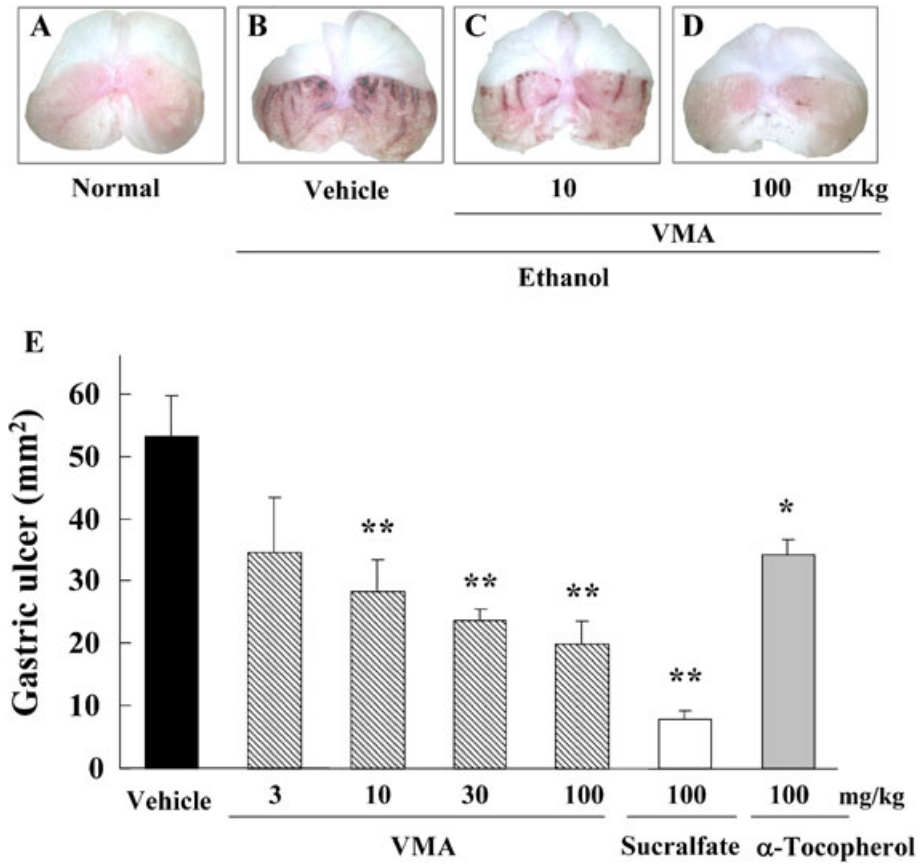

Figure 1. The protective effects of VMA on HCl/ethanol-induced gastric ulcer in mice. Normal stomach (A), glandular stomach treated with vehicle (B), VMA (10 mg/kg, p.o.) (C) and VMA (100 mg/kg, p.o.) (D) 1 h before administration of 150 mM HCl/ethanol. Quantitative analysis of VMA, sucralfate, and α-tocopherol on the ulcer area (mm^2) induced by HCl/ethanol (E). Data are shown as mean ± SEM, $n = 6$ to 8, $^*p < 0.05$, $^{**}p < 0.01$ vs vehicle-treated group, Dunnett's multiple comparison test or Student's *t*-test. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr

ethanol administration inhibited the gastric erosions in a dose-dependent manner (Fig. 1C, D). Additionally, pretreatment of sucralfate (100 mg/kg, p.o.) and α-tocopherol (100 mg/kg, p.o.) significantly reduced the gastric erosions (respective, $p < 0.01$, $p < 0.05$ vs vehicle mice) (Fig. 1E).

The protective effects of VMA were confirmed histologically. In vehicle-treated mouse stomachs, administering HCl/ethanol resulted in large areas of epithelial crypt loss, predominantly neutrophilic infiltrate throughout the mucosa erosion, compared with sham-treated mice (Fig. 2A, B). By contrast, pretreatment with VMA resulted in smaller erosions with few neutrophils (Fig. 2C, D). In the gastric erosions, desquamate mucosal cells were observed in the HCl/ethanol plus vehicle-treated mice (Fig. 2F) compared with sham-treated mice (Fig. 2E). Pretreatment with VMA (10 and 100 mg/kg, p.o.) 1 h before administering HCl/ethanol reduced desquamated mucosal cells in the glandular stomach of mice (Fig. 2G, H).

Lipid peroxidation in murine stomach tissue homogenates

Because gastric ulcer was causally related to lipid peroxidation in the glandular stomach, the effects of VMA on lipid peroxidation were investigated in the murine stomach tissue homogenates. The TBARS level in the murine stomach tissue homogenates was elevated by 60 min incubation with $FeSO_4$ at 37 °C (Fig. 3). The VMA inhibited the production of TBARS in a concentration-dependent manner, with the IC_{50} value being 4.0 μg/mL (95% confidence limits: 1.7–10.3 μg/mL) (Fig. 3).

Radical scavenging activity

Vaccinium myrtillus L. anthocyanoside, delphinidin, cyanidin and malvidin were measured for their superoxide anion radical and hydroxyl radical scavenging activity using ESR spectroscopy. The VMA showed strong radical scavenging activity against the superoxide anion radical. Additionally, delphinidin, cyanidin, and malvidin showed superoxide anion radical scavenging activity in a concentration-dependent manner (Fig. 4A), with decreasing IC_{50} values in the order of malvidin > delphinidin > cyanidin > trolox (Table 1). Against the hydroxyl radical, VMA showed radical scavenging activity in a concentration-dependent manner. Delphinidin and trolox (a water-soluble derivative of vitamin E) also showed hydroxyl radical scavenging activity, but cyanidin and malvidin did not (Fig. 4B, Table 2). VMA and its main anthocyanidins showed more radical scavenging activity against the superoxide anion radical than against the hydroxyl radical.

DISCUSSION

In a previous study, VMA at doses of 200 and 400 mg/kg showed significant inhibitory effects (36% and 24%,

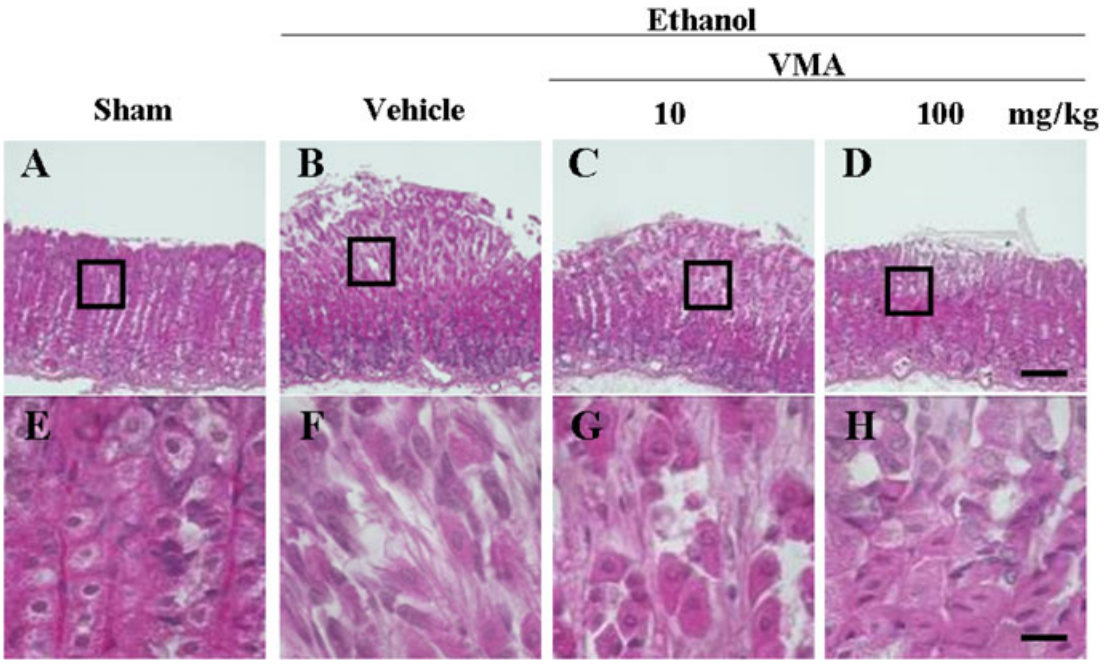

Figure 2. The protective effects of VMA on histological evaluation in an HCl/ethanol-induced ulcer model. Sections of hematoxylin and eosin (HE) staining. Section through gastric mucosa in the sham-treated group (A). Microscopic appearance of lesions induced by HCl/ethanol in gastric mucosa pretreated with vehicle (B), 10 mg/kg (C) and 100 mg/kg (D) of VMA. Scale bars show 150 μm (upper micrograph) and 10 μm (lower micrograph). This figure is available in colour online at wileyonlinelibrary.com/journal/ptr

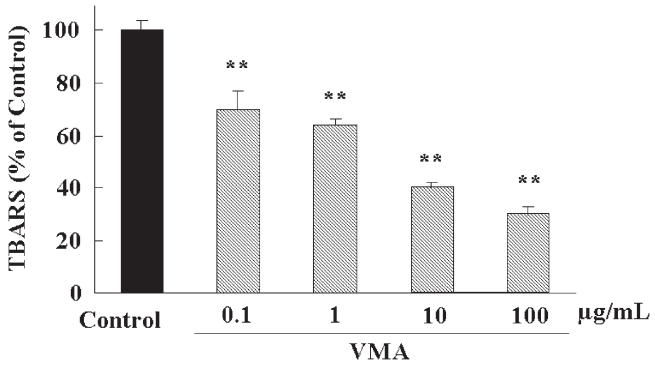

Figure 3. The inhibition effects of VMA against production of TBARS in the murine stomach tissue homogenates. The values of TBARS are shown at a rate of control. Data are shown as mean ± SEM, $n = 3$, $^{**}p < 0.01$ vs control. TBARS, thiobarbituric acid reactive substances.

respectively) against ethanol ulcer lesions in rats, but not dose-related protection (Magistretti *et al.*, 1988). The present study investigated the effects of VMA against HCl/ethanol-induced ulcer lesions in mice. The VMA at doses of 10, 100 and 300 mg/kg showed significant inhibition of gastric lesions (47%, 56% and 63%, respectively) in a dose-dependent manner. These results indicate that VMA may be more protective against the gastric ulcer model of mice than that of rats. Pretreatment with sucralfate and α-tocopherol, which were used as positive controls (mucosal protective antiulcer drug and antioxidant, respectively), also significantly prevented the gastric mucosal injury induced by HCl/ethanol.

VMA has been reported to inhibit lipid peroxidation in various tissues, such as the liver, brain, and kidney (Bao *et al.*, 2008; Rahman *et al.*, 2008; Matsunaga *et al.*, 2009). In the present work, an *in vitro* study was performed to evaluate the inhibitory effects of VMA against the elevation of lipid peroxidation in murine stomach tissue homogenates. VMA significantly decreased the production of lipid peroxidant in murine stomach tissue homogenates in a concentration-dependent manner. High concentrations of ethanol induce lipid peroxidation in stomach tissue through excess superoxide anion radicals generated by the reaction of xanthine oxidase with hypoxanthine (Novak *et al.*, 1991; Huh *et al.*, 1996) and the hydroxyl radical generated by the Fenton reaction. Superoxide anion radicals react with superoxide dismutase (SOD) and are converted to hydrogen peroxide. Antioxidants (such as vitamin E, ascorbic acid, quercetin and tea catechin) are known to reduce lipid peroxidants and to inhibit the oxidation of phospholipids on cell membranes. The present study showed the protective effects of α-tocopherol, an analog of vitamin E, against HCl/ethanol-induced gastric ulcers in mice.

VMA has been reported to exhibit radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH), oxygen radical absorbance capacity (ORAC), the superoxide anion radical and the hydroxyl radical (Prior *et al.*, 1998; Martín-Aragón *et al.*, 1998; Faria *et al.*, 2005). In the present study, ESR was used to show

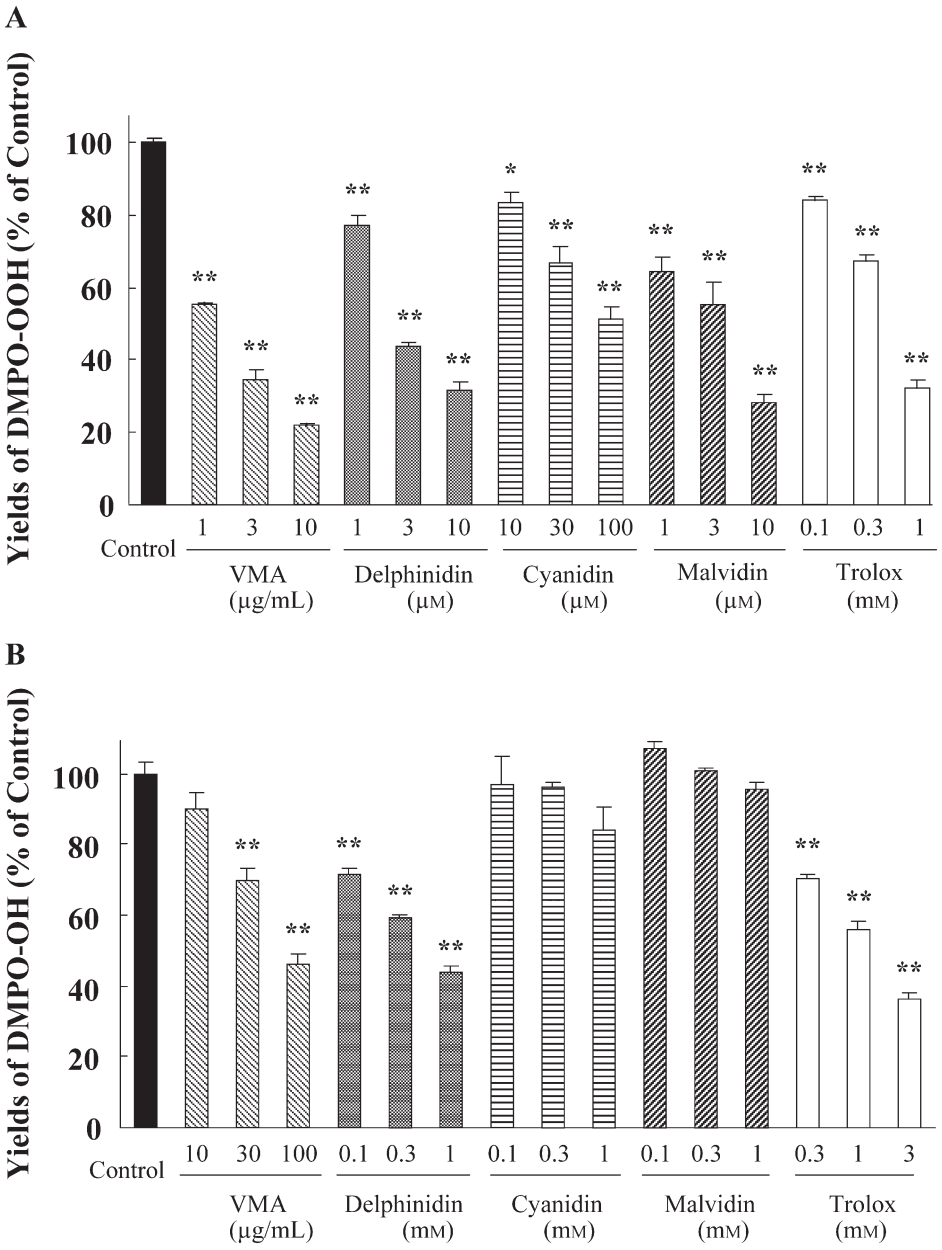

Figure 4. The radical scavenging activities of VMA and its main anthocyanidins against the superoxide anion radical and the hydroxyl radical. VMA, delphinidin, cyanidin, malvidin and trolox were measured for superoxide anion radical scavenging activity (A) and hydroxyl radical scavenging activity (B) by ESR. The values of radical (DMPO-OOH and DMPO-OH) are shown at a rate of control. Data are shown as mean ± SEM, $n = 3$. *$p < 0.05$, **p < 0.01 vs control.

Table 1. Superoxide anion radical scavenging activity of VMA and its main anthocyanidins (delphinidin, cyanidin and malvidin) measured by ESR

Compound	IC_{50}			
	μg/mL		μM	
VMA	1.2	(1.0–1.5)		
Delphinidin	1.2	(0.9–1.6)	3.5	(2.5–4.7)
Cyanidin	31.8	(21.8–50.9)	98.4	(67.5–157.8)
Malvidin	1.0	(0.7–1.4)	2.8	(2.0–3.8)
Trolox	130.8	(113.4–154.0)	522.4	(453.1–615.1)

IC_{50}, 50% inhibitory concentration; VMA, *Vaccinium myrtillus* L. anthocyanin.
The parentheses show 95% confidence limits.

Table 2. Hydroxyl radical scavenging activity of VMA and its main anthocyanidins (delphinidin, cyanidin and malvidin) measured by ESR

Compound	IC_{50}			
	μg/mL		mM	
VMA	116	(80–192)		
Delphinidin	237	(203–305)	0.7	(0.6–0.9)
Cyanidin	>323	>1.0		
Malvidin	>367	>1.0		
Trolox	325	(275–400)	1.3	(1.1–1.6)

IC_{50}, 50% inhibitory concentration; VMA, *Vaccinium myrtillus* L. anthocyanin.
The parentheses show 95% confidence limits.

that VMA possesses strong radical scavenging activity against the superoxide anion radical generated by the xanthine oxidase reaction and the hydroxyl radical generated by the Fenton reaction. Although VMA has been reported to show radical scavenging activity against the superoxide anion radical when measured with ESR (Rahman *et al*., 2006), this is the first report to investigate the effect of VMA against the hydroxyl radical. Delphinidin, cyanidin and malvidin (three main anthocyanidins in VMA) showed radical scavenging activity against the superoxide anion radical. Additionally, delphinidin, but not cyanidin or malvidin, showed hydroxyl radical scavenging activity. These results suggest the hydroxyl radical scavenging effects of VMA may contribute mainly to that of delphinidin.

Ethanol treatment decreases the levels of glutathione, SOD and catalase, which convert the hydroxyl radical to H_2O and oxygen (Parks, 1989). Oral administration of cyanidin-3-glucoside (4 and 8 mg/kg, p.o.) has been reported to inhibit the reduction of glutathione, SOD and catalase, induced by ethanol treatment in the rat stomach (Li *et al*., 2008). In addition, cyanidin-3-glucoside inhibits the elevation of lipid peroxidant levels in rat stomach tissue after ethanol treatment (Li *et al*., 2008). In the present study, VMA, containing cyanidin-3-glucoside, might inhibit the reduction of each antioxidative enzyme and the production of lipid peroxidant in murine stomach.

In conclusion, these findings indicate that VMA and its main anthocyanidins (delphinidin, cyanidin and malvidin) show protective effects against HCl/ethanol-induced gastric ulcer in mice, presumably due to their radical scavenging effects and antioxidative effects.

Conflict of Interest

The authors have declared that there is no conflict of interest.

REFERENCES

Bao L, Yao XS, Tsi D *et al.* 2008. Protective effects of bilberry (*Vaccinium myrtillus* L.) extract on $KBrO_3$-induced kidney damage in mice. *J Agric Food Chem* **56**: 420–425.

Colantuoni A, Bertuglia S, Magistretti MJ, Donato L. 1991. Effects of *Vaccinium myrtillus* anthocyanosides on arterial vasomotion. *Arzneimittelforschung* **41**: 905–909.

Das D, Banerjee RK. 1993. Effect of stress on the antioxidant enzymes and gastric ulceration. *Mol Cell Biochem* **125**: 115–125.

Del Maestro RF, Bjork J, Arfors KE. 1981. Increase in microvascular permeability induced by enzymatically generated free radicals. I. *In vivo* study. *Microvasc Res* **22**: 239–254.

Del Soldato P, Foschi D, Benoni G, Scarpignato C. 1986. Oxygen free radicals interact with indomethacin to cause gastrointestinal injury. *Agents Actions* **17**: 484–488.

Eastwood GL, Kirchner JP. 1974. Changes in the fine structure of mouse gastric epithelium produced by ethanol and urea. *Gastroenterology* **67**: 71–84.

Faria A, Oliveira J, Neves P *et al.* 2005. Antioxidant properties of prepared blueberry (*Vaccinium myrtillus*) extracts. *J Agric Food Chem* **53**: 6896–6902.

Hamaishi K, Kojima R, Ito M. 2006. Anti-ulcer effect of tea catechin in rats. *Biol Pharm Bull* **29**: 2206–2213.

Huh K, Shin US, Lee SH. 1996. The effect of rebamipide on gastric xanthine oxidase activity and type conversion in ethanol-treated rats. *Free Radic Biol Med* **20**: 967–971.

Jaarin K, Renuvathani M, Nafeeza MI, Gapor MT. 1999. Comparative effect of palm vitamin E and ranitidine on the healing of ethanol-induced gastric lesions in rats. *Int J Exp Pathol* **80**: 259–263.

Jeong-Hwan Kim S-KC, Sang-Yun Choi, Han-Kyeom Kim, Hyo-Ihl Chang. 2005. Suppressive effect of astaxanthin isolated from the *Xanthophyllomyces dendrorhous* mutant on ethanol-induced gastric mucosal injury in rats. *Biosci Biotechnol Biochem* **69**: 1300–1305.

Li CY, Xu HD, Zhao BT, Chang HI, Rhee HI. 2008. Gastroprotective effect of cyanidin 3-glucoside on ethanol-induced gastric lesions in rats. *Alcohol* **42**: 683–687.

Lulu DJ, Dragstedt LR. 1970. Massive bleeding due to acute hemorrhagic gastritis. *Arch Surg* **101**: 550–554.

Magistretti MJ, Conti M, Cristoni A. 1988. Antiulcer activity of an anthocyanidin from *Vaccinium myrtillus*. *Arzneimittelforschung* **38**: 686–690.

Martín-Aragón S, Basabe B, Benedí JM, Villar AM. 1998. Antioxidant action of *Vaccinium myrtillus* L. *Phytother Res* **12**: 104–106.

Matsunaga N, Imai S, Inokuchi Y *et al.* 2009. Bilberry and its main constituents have neuroprotective effects against retinal neuronal damage *in vitro* and *in vivo*. *Mol Nutr Food Res* **53**: 869–877.

Noda Y, Anzai K, Mori A, Kohno M, Shinmei M, Packer L. 1997. Hydroxyl and superoxide anion radical scavenging activities of natural source antioxidants using the computerized JES-FR30 ESR spectrometer system. *Biochem Mol Biol Int* **42**: 35–44.

Noda Y, Kohno M, Mori A, Packer L. 1999. Automated electron spin resonance free radical detector assays for antioxidant activity in natural extracts. *Methods Enzymol* **299**: 28–34.

Novak L, Stipek S, Zima T, Trojan S. 1991. Plasma xanthine oxidase and resistance to hypoxia: effect of purines and alcohol administration. *Drug Metab Drug Interact* **9**: 311–320.

Ömer C, Mehmet K, Ferah A, Kurtuluş I. 2004. Protective effects of quercetin, a flavonoid antioxidant, in absolute ethanol-induced acute gastric ulcer. *Eur J Gen Med* **1**: 37–42.

Parks DA. 1989. Oxygen radicals: mediators of gastrointestinal pathophysiology. *Gut* **30**: 293–298.

Pihan G, Regillo C, Szabo S. 1987. Free radicals and lipid peroxidation in ethanol- or aspirin-induced gastric mucosal injury. *Dig Dis Sci* **32**: 1395–1401.

Prior RL, Cao G, Martin A *et al.* 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of Vaccinium species. *J Agric Food Chem* **46**: 2686–2693.

Rahman MM, Ichiyanagi T, Komiyama T, Hatano Y, Konishi T. 2006. Superoxide radical- and peroxynitrite-scavenging activity of anthocyanins; structure-activity relationship and their synergism. *Free Radic Res* **40**: 993–1002.

Rahman MM, Ichiyanagi T, Komiyama T, Sato S, Konishi T. 2008. Effects of anthocyanins on psychological stress-induced oxidative stress and neurotransmitter status. *J Agric Food Chem* **56**: 7545–7550.

Rosen GM, Rauckman EJ. 1984. Spin trapping of superoxide and hydroxyl radicals. *Methods Enzymol* **105**: 198–209.

Shaw S, Herbert V, Colman N, Jayatilleke E. 1990. Effect of ethanol-generated free radicals on gastric intrinsic factor and glutathione. *Alcohol* **7**: 153–157.

Szabo S. 1987. Mechanisms of mucosal injury in the stomach and duodenum: time-sequence analysis of morphologic, functional, biochemical and histochemical studies. *Scand J Gastroenterol* **127**: 21–28.

Szelenyi I, Brune K. 1988. Possible role of oxygen free radicals in ethanol-induced gastric mucosal damage in rats. *Dig Dis Sci* **33**: 865–871.

Tariq M. 1988. Gastric anti-ulcer and cytoprotective effect of vitamin E in rats. *Res Commun Chem Pathol Pharmacol* **60**: 87–96.

Tarnawski A, Stachura J, Ivey KJ, Mach T, Bogdal J, Klimczyk B. 1981. Ethanol induced duodenal lesions in man. Protective effect of prostaglandin. *Prostaglandins* **21** (Suppl): 147–153.

Terano A, Hiraishi H, Ota S, Shiga J, Sugimoto T. 1989. Role of superoxide and hydroxyl radicals in rat gastric mucosal injury induced by ethanol. *Gastroenterol Jpn* **24**: 488–493.