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# Effect of pear (*Pyrus communis* L.) procyanidins on gastric lesions induced by HCl/ethanol in rats

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**Abstract**

Effect of pear procyanidins on ethanol-induced gastric ulcer in rats was investigated. In a preliminary experiment, the percentage of lesion area to total gastric surface area (ulcer index) increased with increases in ethanol concentration (40–80%) and the length of time after ethanol treatment (60–120 min). Migration of leukocytes, estimated by myeloperoxidase activity in mucosa, was involved in the occurrence of an ulcer, whereas expansion of the ulcer seemed to be due to leukocytes that had already migrated. Highly polymerized procyanidins extracted from 'Winter Nélis' pear fruit, orally administered (20 mg/rat) before 60% ethanol treatment, exhibited a high level of antiulcer capacity whereas chlorogenic acid alone seemed to have a negative effect. The trend of myeloperoxidase activity was similar to the trend of the ulcer index. A mixture of those polyphenols had a significant protective effect. The results suggest that the antiulcer effect of pear procyanidins may be due to their strong antioxidant activity.


*Keywords:* *Pyrus communis*; Polyphenols; Procyanidin; Chlorogenic acid; Antiulcer activity; Myeloperoxidase; Gastric mucosa injury

## 1. Introduction

Some European pear (*Pyrus communis* L.) cultivars have been reported to contain significant amounts of polyphenols, such as chlorogenic acid, flavan-3-ols and arbutin (Escarpa & Gonzalez, 2000; Oleszek, Amiot, & Aubert, 1994). It is known the astringency is due to the presence of considerable amounts of procyanidins, which comprise a large proportion of the total polyphenols in certain cultivars, such as 'Joséphine de Malines' (Hamauzu & Hanakawa, 2003) or 'Winter Nélis' especially harvested from dwarf trees. Procyanidins are also related to flesh browning of pear fruit (Hamauzu & Hanakawa, 2003). Thus, pear cultivars rich in procyanidins sometimes have undesirable characteristics as a fresh commodity.

Despite some undesirable aspects, procyanidins are reported to have many beneficial properties, such as potent antioxidant activity (Ariga, Koshiyama, & Fukushima, 1988; Lu & Foo, 2000; Teissedre, Frankel, Waterhouse, Peleg, & German, 1996; Zhu, Holt, Lazarus, Orozco, & Keen, 2002), free radical scavenging activity (Ariga & Hamano, 1990; Arteel & Sies, 1999), anti-inflammatory activity (Osawa et al., 1999) and anti-influenza viral activity (Hamauzu, Yasui, Inno, Kume, & Omanyuda, 2005).

Recently, some polyphenolics have been found to have a preventive action on gastric injury in rats. Some research has focussed on the antiulcer activity of polyphenol from grape seed (Saito, Hosoyama, Ariga, Kataoka, & Yamaji, 1998), cacao liquor (Osakabe, Sanbongi, Yamagishi, Takizawa, & Osawa, 1998) or from *Opuntia ficus indica* (Galati et al., 2003). This activity was mainly explained by strong antioxidant power and/or by some other factors, such as

*Abbreviations:* WND, 'Winter Nélis' dwarf; ME, 80% methanol extracts; AE, 60% acetone extracts; MPO, myeloperoxidase; mDP, mean degree of polymerization; UI, ulcer index.

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strong protein-binding capacity (Saito et al., 1998), modulation of leukocyte function (Osakabe et al., 1998), and mucus production and restoration (Galati et al., 2003). The strong protein-binding capacity has been reported to be a characteristic of highly polymerized procyanidins (Saito et al., 1998). It is believed that the antioxidant activity of polyphenols is an important factor because reactive oxygen and/or free radicals are related to the occurrence of ulcers.

Free radicals are involved in several pathological processes, such as inflammation, tissue damage caused by ischemia and reperfusion, radiation, or chemicals (Freeman & Crapo, 1982; Halliwell & Gutteridge, 1985). Oxygen-derived free radicals have been shown to participate in reperfusion damage in the intestine (Parks, Bulkley, Granger, Hamilton, & McCord, 1982; Parks & Granger, 1983; Parks, Shah, & Granger, 1984) and in the stomach (Itoh & Guth, 1985). More recently, HCl/ethanol has been used to induce gastric lesions in rats as a model. The exact mechanism of pathogenesis by HCl/ethanol is still unclear (Matsumoto, Moriguchi, & Yamada, 1993), but it has been demonstrated that active oxygen species might be involved in the formation of gastric mucosal lesions (Itoh & Guth, 1985; Smith, Grisham, Manci, Granger, & Kvietys, 1987; Yoshikawa et al., 1990). Enzymatic antioxidants, such as superoxide dismutase (SOD), catalase, and combinations of those, are effective in prevention of ulcers induced by ischemia-reperfusion (Tanaka & Yuda, 1993; Yoshikawa et al., 1989), acute stress (Alican et al., 1994; Yoshikawa, Miyagawa, Yoshida, Sugino, & Kondo, 1986), and ethanol (Matsumoto et al., 1993; Pihan, Regillo, & Szabo, 1987; Szelenyi & Brune, 1988).

The objective of this study was to investigate the effect of pear polyphenols (especially procyanidins) on ethanol-induced gastric lesions in rats.

## 2. Materials and methods

### 2.1. Plant material

'Winter Nélis' dwarf (WND) trees, grafted on ''Old Home'' (inter-stock)/quince C (rootstock), were grown at the Education and Research Center of Alpine Field Science, Faculty of Agriculture, Shinshu University, Japan. Fruits at the fully mature stage were used for experiments. The flesh was cut into small pieces, frozen in liquid $N_2$ and freeze-dried using an EYELA FD-5N freeze dryer (Tokyo Rikakikai Co., Ltd., Tokyo, Japan). Then samples were put into powder using a mixer and stored at −18 °C in a desiccator for further use.

### 2.2. Solvents and reagents

Chlorogenic acid standard was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Solvents were purchased from Nacalai Tesque and TMB (3,3′,5,5′-tetra-methyl-benzidine) was from Moss, Inc. (Pasadena, MD, USA). Thirty percent Hydrogen peroxide solution was purchased from Santoku Chemical industry Co., Ltd. (Tokyo, Japan). Folin–Ciocalteu reagent and toluene-α-thiol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

### 2.3. Preparation of pear procyanidins

Polyphenols were extracted from freeze-dried pear flesh using 80% methanol, twice, followed by use of 60% acetone, twice. Both 80% methanol extract (ME) and 60% acetone extract (AE) were analyzed using HPLC for evaluation of phenolic composition. Total phenolic content of extracts was estimated by the Folin–Ciocalteu method, using Folin–Ciocalteu reagent, as described below. The AE (containing mainly procyanidin polymers) was concentrated under-vacuum, washed with petroleum ether, and freeze-dried for further use.

### 2.4. Determination of total phenolics

The experimental procedure was adapted from those of Hamauzu et al. (2005). Two ml of sample solution were mixed with 2 ml of Folin–Ciocalteu reagent in a test tube. After 3 min of reaction, 2 ml of $Na_2CO_3$ (10 g/100 ml) were added and the mixture was incubated for 60 min at room temperature. The absorbance was measured at 700 nm with a Shimadzu UV-1200 spectrophotometer (Tokyo, Japan) against a blank (2 ml of deionized water, plus reagents) in the reference cell. (−)-Epicatechin was used as the standard.

### 2.5. HPLC analysis of phenolic extracts

Chromatographic separation was carried out on a Luna 5 μ C18 column (150 × 4.6 mm, Phenomenex, Inc., Torrance, CA., USA) at 40 °C using two solvents: 0.1% aqueous phosphoric acid (A) and 0.1% phosphoric acid in acetonitrile (B). The gradient programme was started with 5% B and changed to 15% B at 30 min, 32% B at 35 min, 40% B at 45 min, and 75% B at 50 min. The 75% B was maintained until 65 min. The flow rate was 1.0 ml/min and the injection volume was 20 μl. Detection was performed at 280 and 325 nm on a Shimadzu SPD-M10Avp photodiode array detector. Identification of pear phenolics was achieved by comparing retention times and UV spectra with those of standards. Thioacidolysis, described below, was also used to get information for peak identification.

### 2.6. Estimation of the mean degree of polymerization of procyanidins

The mean degree of polymerization was determined by calculating the molar ratio of all flavan-3-ol units (thioether adducts plus terminal units) to (−)-epicatechin and (+)-catechin, corresponding to terminal units after thioacidolysis, as described in Guyot, Marnet, Laraba, San-

oner, and Drilleau (1998). The experimental procedures were adapted from that of Hamauzu et al. (2005). The standard curve for (−)-epicatechin benzyl thioether was made by thioacidolysis of the procyanidin $B_2$ standard.

### 2.7. Antiulcer test

#### 2.7.1. Animals

Male Wistar rats (Jcl:Wistar), weighing 230–262 g, were obtained from CLEA Japan, Inc., (Tokyo), kept in a controlled environment (temperature 20 ± 5 °C; humidity 55 ± 10%; 12-h light-dark cycle), and maintained on a standard diet (CE-2, CLEA Japan, Inc.) for 3 d. The animals were fasted 24 h before the experiment and allowed free access to water. The experiments were approved by the ethics committee of Shinshu University.

#### 2.7.2. Treatment

Rats were administered doses of 1.5 ml/rat (containing 20 mg of polyphenol) of test solution intragastrically and then given 1.5 ml/rat of acidified ethanol solution (150 mM HCl/ethanol = 40:60 v/v) after 30 min. For control rats, 1.5 ml of water was given instead of the polyphenolic solution. Animals were sacrificed under anesthesia 60 min after HCl/ethanol administration. In order to understand the mechanism of ulceration and choose suitable conditions for subsequent experiments, different concentrations of ethanol (40%, 60% and 80%) and different times of exposure to ethanol (60, 90 and 120 min) were also investigated. Stomachs were removed, opened along the greater curvature and rinsed with physiological saline. They were stretched on balsa boards and pinned with the mucosal side up. After taking digital pictures of the mucosal surface of each stomach for morphometrical analysis, as described below, stomachs were cut along the lesser curvature with razor blades and divided in half. One half was frozen with liquid nitrogen and kept at −20 °C under nitrogen gas for further evaluation of myeloperoxidase activity in the mucosa. The other half was processed for histological analysis as described below.

#### 2.7.3. Lesions analysis

The degree of gastric mucosal damage was evaluated from digital pictures, using a computerized image analysis system (Zeiss, KS400, Göttingen, Germany). Percentage of the total lesion area (hemorrhage sites) to the total surface area of the stomach, except the forestomach, was defined as the ulcer index (UI).

#### 2.7.4. Histological analysis

After digital pictures of each stomach were obtained, tissues were immediately immersed in the fixative, 4% paraformaldehyde, in phosphate buffer (pH 7.6), for 48 h at 4 °C. Paraffin sections, 5 μm thick, were made and stained with Mayer's hematoxylin and eosin according to standard procedures. Tissue preparations were observed and microphotographed under a light microscope.

### 2.8. Measurement of myeloperoxidase activity

#### 2.8.1. Preparation of crude enzyme from stomach mucosa

From the frozen samples, small pieces of mucosa were taken using a razor blade to obtain a random sample of approximately 50 mg of mucosa. Those 50 mg were then homogenized in 5 ml of 0.2 M acetate buffer (pH 5) using a potter homogenizer. The homogenates were used to evaluate protein concentration and myeloperoxidase activity.

#### 2.8.2. Protein concentration in mucosa

The concentration of protein in the homogenized mucosa solution was determined by the Bradford method (Bradford, 1976), using bovine serum albumin (BSA) as a standard. One millilitre of Bradford reagent was added to 100 μl of the diluted sample. Absorbance was measured at 595 nm. Protein concentrations were expressed in mg/l (A).

#### 2.8.3. Myeloperoxidase activity

Activity of MPO, a marker enzyme of leukocytes, is thought to represent leukocyte migration to the injured tissues. The reaction mixture consisted of 117 mM acetate buffer (pH 5), 0.4 mM tetramethylbenzidine (TMB), 0.3% $H_2O_2$ and crude enzyme solution. Absorbance at 655 nm (A655) of the mixture was immediately recorded for 5 min. Activity (U) was calculated from the increase in optical density per min (ΔA655/min) (B) and finally expressed as activity per mg protein as follows:

$$\text{Myeloperoxidase activity (U/mg protein)} = (\text{B}) \times 1000/(\text{A}).$$

### 2.9. Statistical analysis

Results are expressed as means ± SE. Means were compared by Fisher's protected LSD multiple comparison test (for ulcer index) or Scheffe's test (for MPO activity) using Excel 2000 with the add-in software Statcel 2 (OMS, Tokyo, Japan). Simple comparison between the data of control group and that of test group was also performed, using the Student's *t*-test. Differences were considered significant at $P < 0.05$.

## 3. Results

### 3.1. Polyphenols from pear

Total polyphenol content of WND fruit in ME and AE, assessed by the Folin–Ciocalteu method, was 15.1 mg/100 gFW and 51.2 mg/100 gFW, respectively (Table 1). The main phenolic compound in ME was chlorogenic acid (approx. 82%) and a small amount of other phenolics was also detected by HPLC analysis (Fig. 1(A)). Polyphenols in AE were principally procyanidins, found in large

Table 1
Total phenolics, chlorogenic acid content and mean degree of polymerization of procyanidins extracted with 80% methanol and 60% acetone successively from 'Winter Nelis' pear fruit

| Fraction | Total phenolics[a] | Chlorogenic acid | Others | mDP[b] |
|---|---|---|---|---|
| ME | 15.1 (1.2) | 12.4 (1.03) | 2.7 (0.3) | 6.9 (0.5) |
| AE | 51.2 (5.0) | 0.2 (0.02) | 51.0 (5.0) | 88.8 (4.2) |

Data are mean (SE) of three assays.
Abbreviations: ME, 80% methanol extract; AE, 60% acetone extract.
[a] Values are expressed in mg/100 gFW by the Folin–Ciocalteu method.
[b] mDP, mean degree of polymerization of procyanidins.

amounts (Fig. 1(B) and Table 1), and thioacidolysis revealed that they had a quite high mean degree of polymerization (Fig. 1(C) and Table 1).

### 3.2. Effect of ethanol concentration and exposure time on induction of gastric ulcer

By macroscopic observation, the acute ulcer induced by HCl/ethanol appeared to have intense gastric hyperemia, extending in a band-like conformation, and consisted of thickened lesions as well as many filiform lesions. Most of the lesions were black or reddish-brown. Stomach treated with 80% ethanol was particularly damaged, having severe edema and a disgusting "gelatine" aspect. Fig. 2 shows that, with increasing concentrations of ethanol, the surface of the lesion and MPO activity in the mucosa increased. The UI of stomach treated with 40%, 60% and 80% ethanol were 5.1, 8.7 and 18.9, respectively, and MPO activities (U/mg protein) were 12.9, 20.4 and 28.7,

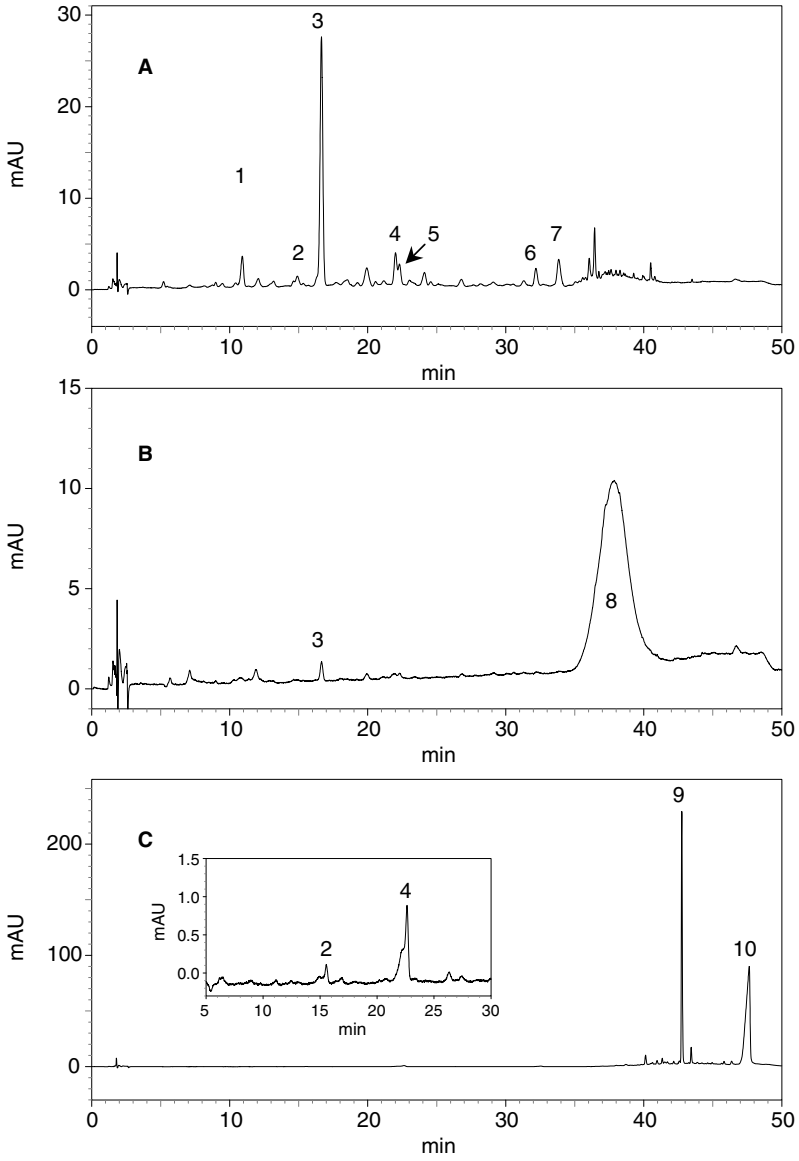


Fig. 1. HPLC chromatogram of phenolics in 80% methanol fraction (A) and 60% acetone fraction (B) from 'Winter Nelis' pear fruit. Chromatogram (C) shows thioacidolysis products of 60% acetone extracts. Peaks: 1, unidentified; 2, (+)-catechin; 3, chlorogenic acid; 4, (−)-epicatechin; 5 and 6, hydroxycinnamic derivatives; 7, unidentified; 8, polymeric procyanidins; 9, (−)-epicatechin benzyl thioether; 10, toluene-α-thiol. Detection was at 280 nm.

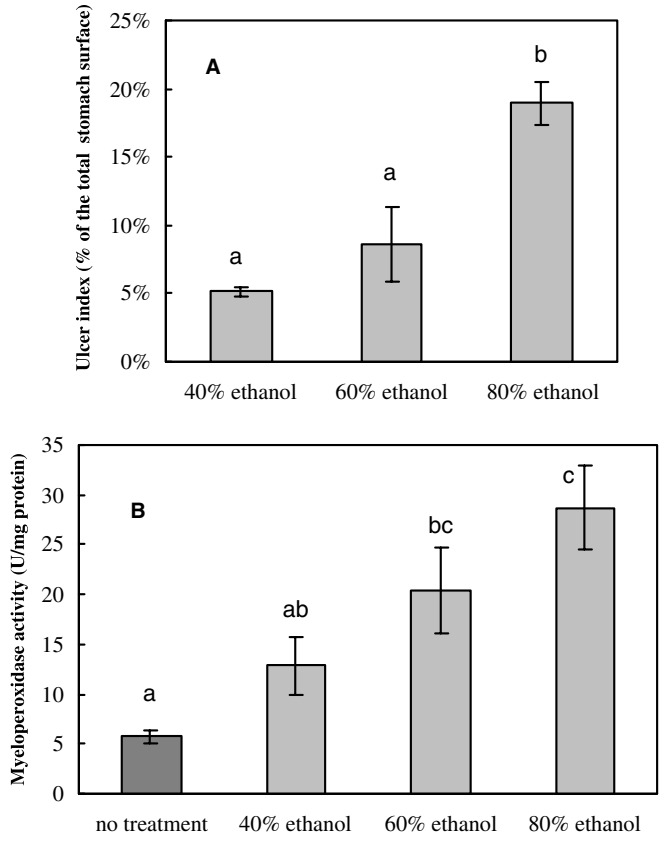


Fig. 2. Effect of the ethanol concentration on lesion surface (A) and myeloperoxidase activity (B) of rat mucosa. Bars indicate SE ($n = 3$). Values with different letters are significantly different at $P < 0.05$.

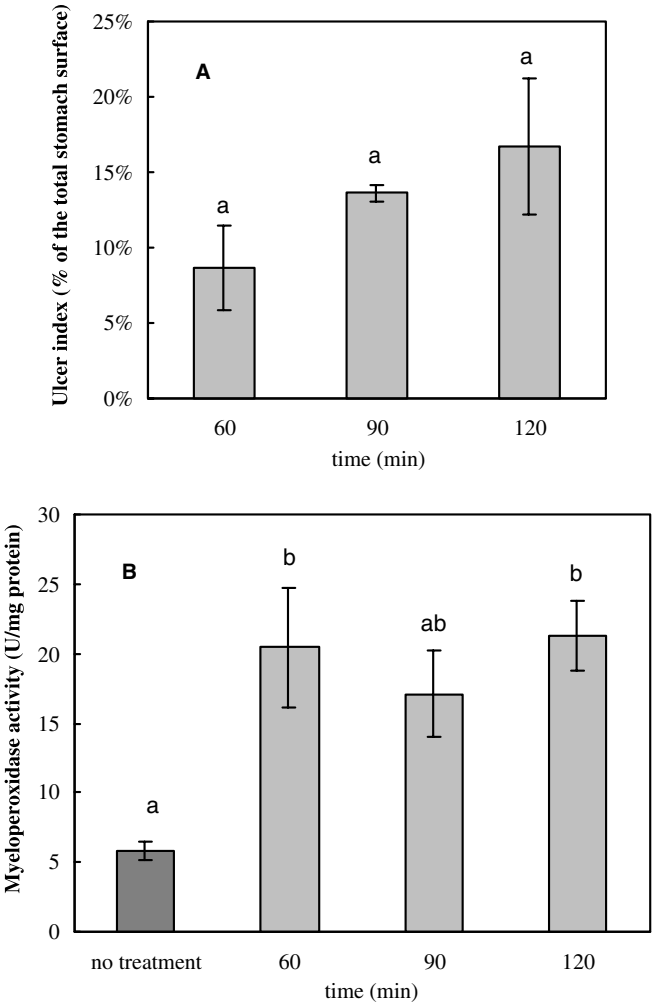


Fig. 3. Effect of the duration of contact between ethanol and mucosa on lesion surface (A) and myeloperoxidase activity (B). Bars indicate SE ($n = 3$). Values with different letters are significantly different at $P < 0.05$.

respectively. In comparison, MPO activity in the normal rat was 5.8. As for duration, the lesion surface tended to increase with increases in the duration of contact of the mucosa with ethanol (UI of stomach exposed to 60% ethanol for 60, 90, and 120 min were 8.7, 13.6, and 16.7, respectively). On the other hand, MPO activity remained almost the same over those time periods (activity was 17.1–21.3 U/mg protein, Fig. 3). From these results, the use of 60% ethanol concentration and a duration of 60 min were selected as the initial conditions for the following ethanol-induced ulcer experiments.

### 3.3. Effect of pear polyphenols on gastric ulcer

The effect of procyanidins from pears has been compared with that of chlorogenic acid, one of the major polyphenols in pears (Fig. 4). Interestingly, chlorogenic acid, which acts as an antioxidant, has no protective effect on ulcers induced by ethanol. Moreover, the lesion surface was even larger with chlorogenic acid (UI was 12.5 for the stomach pretreated with chlorogenic acid vs. 4.1 for that of control) and MPO activity showed a similar trend. Contrary to the result obtained with 80% ethanol, where the lesion was deeper and more severe, observation of the lesion that occurred when using chlorogenic acid and 60% ethanol showed that the surface area of hemorrhage had widened (Fig. 5). As for procyanidins from pear, these appeared to protect the stomach very effectively. There was almost no visible lesion in any tested rat (UI = 0.09) and MPO activity was similar to that in normal stomach. Activity was 7.0 U/mg protein for the stomach pretreated with pear procyanidins and 5.8 U/mg protein for normal stomach. Strong binding of procyanidins to the mucosa was observed during the experiment (visible on Fig. 5).

To determine the potential effect of whole, or natural, pear polyphenols, a mixture of 10 mg chlorogenic acid and 10 mg pear procyanidins was given to rats, following the same method. Table 2 shows that the mixture solution was very effective in protecting mucosa from gastric lesions (UI = 0.61). Procyanidins from pear seemed to compensate for the negative effect of chlorogenic acid.

### 3.4. Histological analysis

Stomach treated with 60% ethanol manifested necrosis of surface mucous cells and fundus glands, hemorrhage

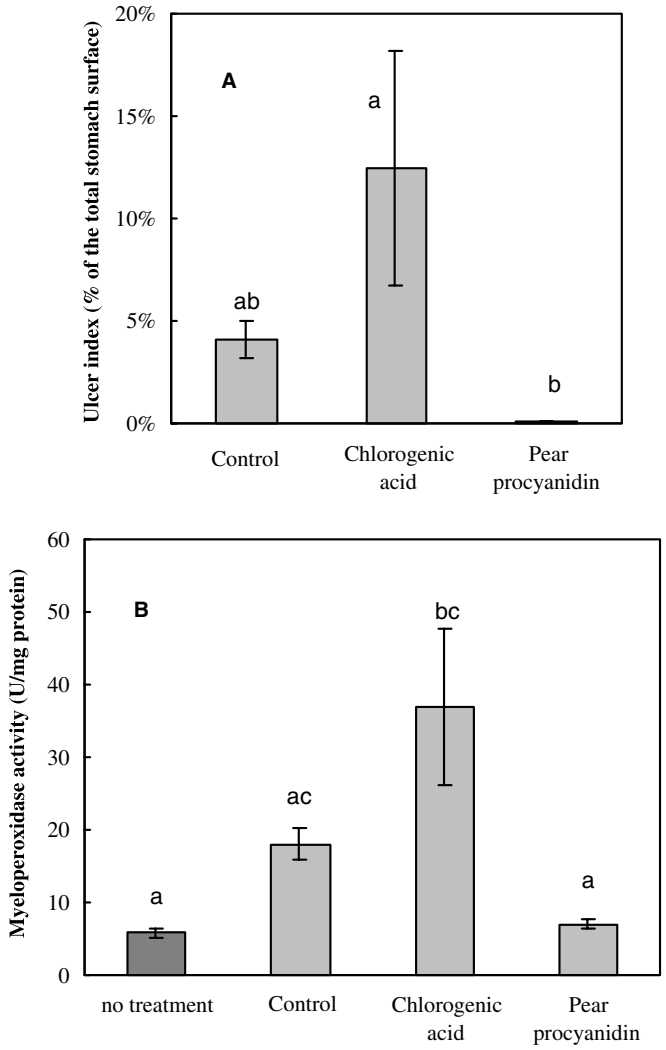


Fig. 4. Effect of procyanidins from pear and chlorogenic acid on lesion surface (A) and myeloperoxidase activity (B). Control, 60% ethanol treatment after administration of water; Chlorogenic acid, 20 mg of chlorogenic acid followed by 60% ethanol; Pear procyanidins, 20 mg of procyanidin followed by 60% ethanol. Bars indicate SE ($n = 5$). Values with different letters are significantly different at $P < 0.05$.

Table 2
Effect of pear procyanidins, chlorogenic acid and their mixture on gastric lesion surface induced by HCl/60% ethanol

| Treatment[a] | Ulcer index[b] |
|---|---|
| Control | 4.11 (0.91) |
| Chlorogenic acid | 12.46 (5.76) |
| Pear procyanidins | 0.09 (0.04)** |
| Mixture | 0.61 (0.38)** |

[a] Treatment: Control, 60% ethanol treatment after administration of water; Chlorogenic acid, administration of 20 mg of chlorogenic acid followed by 60% ethanol; Pear procyanidins, administration of 20 mg of pear procyanidins followed by 60% ethanol; Mixture, administration of 10 mg of chlorogenic acid + 10 mg of pear procyanidins followed by 60% ethanol.

[b] Ulcer index is expressed in percent of the lesion area to the total stomach surface area as mean (SE) of five rats.

** $P < 0.01$ (vs. control) by Student's $t$-test.

in the mucosal layer and edema in the submucosal layer (Fig. 6(A)). Necrosis extended to the body of the fundus glands. Surface mucous cells were pyknotic and desquamated from mucosa. Gastric mucosa of rats treated with pear procyanidins was almost normal in appearance (Fig. 6(B)). Necrosis of surface mucous cells was rarely observed and if noted was not severe. Fundus glands were not affected by ethanol treatment. Injury to the gastric mucosa was exacerbated by treatment with chlorogenic acid (Fig. 6(C)). Necrosis of fundus glands extended to muscularis mucosae and hemorrhage was observed extensively over the mucosa.

## 4. Discussion

### 4.1. General

The focusses of our research were, first, to investigate HCl/ethanol-induced gastric ulcer in rats from the aspect of MPO activity in mucosa, and, second, to assess the preventive effect of pear polyphenols using this evaluation system.

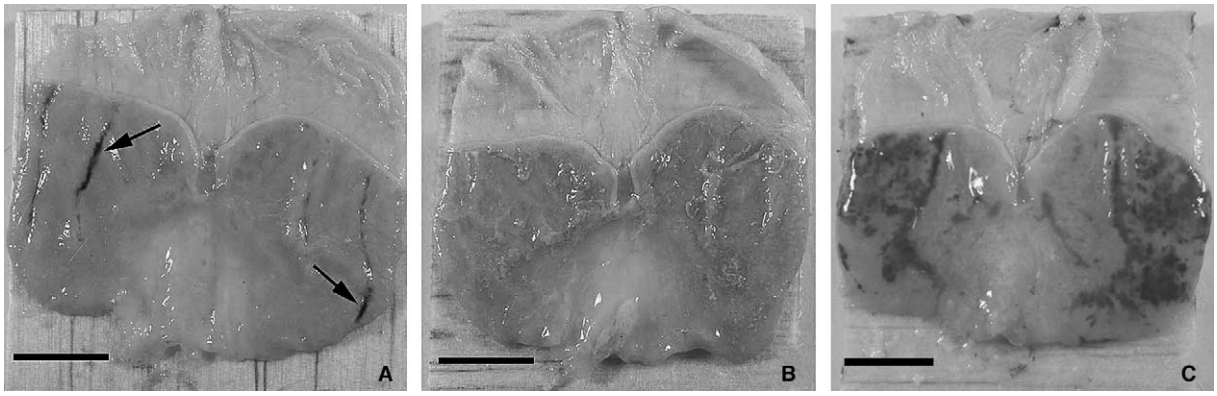


Fig. 5. Photomicrographs showing mucosal surface of rat stomach. (A) Stomach from rat treated with 60% ethanol. Arrows indicate hemorrhage sites. (B): Stomach from rat treated with pear procyanidins + 60% ethanol. (C): Rat treated with chlorogenic acid + 60% ethanol. Hemorrhage sites were observed extensively in comparison with (A). Bar: 1 cm.

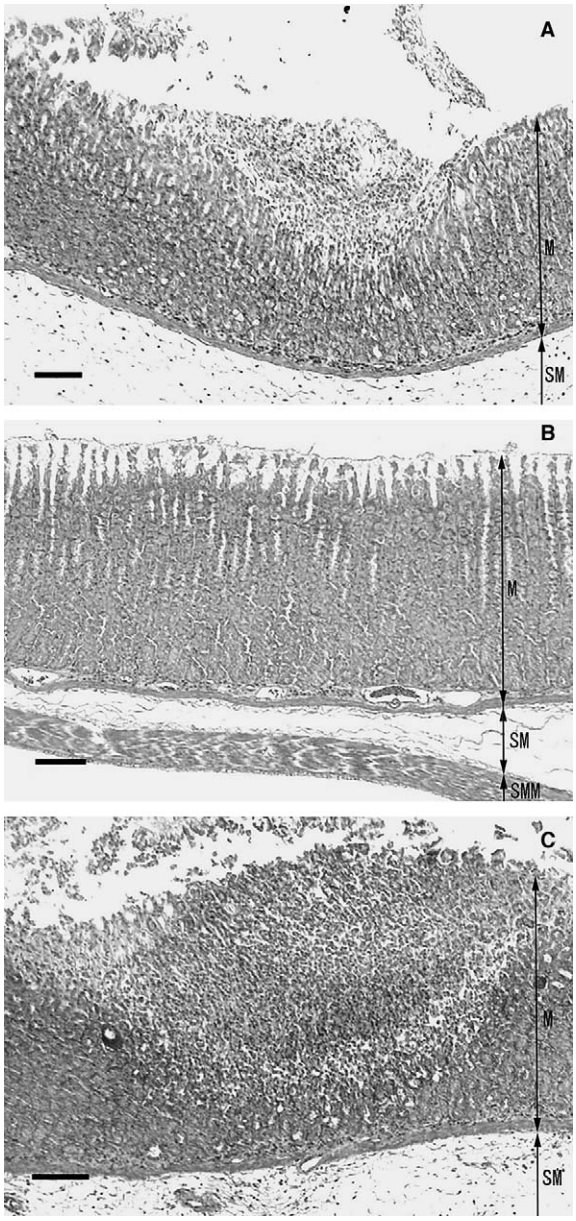


Fig. 6. Histological findings of rat gastric mucosa. (A) Stomach from rat treated with 60% ethanol. Necrosis of surface mucous cells and fundus gland, hemorrhage in mucosal layer and edema of submucosal layer are observed. (B) Stomach from rat treated with pear procyanidins + 60% ethanol. No obvious injuries are recognized in mucosal layer. (C) Rat treated with chlorogenic acid + 60% ethanol. Exacerbation of injury is recognized in comparison with (A). Necrosis of fundus glands reached muscularis mucosae and hemorrhage was observed extensively in mucosa. M, mucosal layer; SM, submucosal layer; SMM, smooth muscle layer. Bar: 100 μm.

### 4.2. HCl/ethanol-induced ulcer mechanism

Although the exact mechanism of pathogenesis by HCl/ethanol is not well understand, recent studies have demonstrated that active oxygen species might be involved in gastric mucosal damage. To confirm the role of free radicals in the process of ulceration, various experiments have been conducted using enzymatic antioxidants such as SOD and catalase (Matsumoto et al., 1993; Pihan et al., 1987). Results showed that the injury to the gastric mucosa induced by HCl/ethanol was significantly inhibited by treatment with those enzymes. Mannitol, a non-enzymatic oxygen radical scavenger, also significantly prevented mucosal injury.

Much of the work on the source of active oxygen radicals in gastric lesions has focussed on xanthine oxidase (XOD) (Itoh & Guth, 1985; Perry, Wadhwa, Parks, Pickard, & Granger, 1986). However, the possibility that XOD is not the main source of radicals has been suggested (Grisham, Hernandez, & Granger, 1986; Hernandez et al., 1987; Tanaka & Yuda, 1993). Xanthine oxidase-derived oxy-radicals appear to activate and attract neutrophils into the mucosa, and these activated neutrophils subsequently injure the microvasculature via release of oxy-radicals or proteases (Hernandez et al., 1987). Matsumoto et al. (1993) used allopurinol, a competitive inhibitor of xanthine oxidase, to show that XOD was not the main source of free radicals. Another study (Osakabe et al., 1998) showed that ethanol treatment did not increase XOD activity.

However, an experiment (Matsumoto et al., 1993) using anti-neutrophil serum showed that oxygen radicals, generated from neutrophils, have an important role in formation of gastric lesions. The mechanism by which HCl/ethanol activates neutrophils is unclear. Because XOD seems not to be the main source of free radicals in the ethanol-induced ulcer system, recent research (Osakabe et al., 1998) has focussed on migration of leukocytes that produce oxygen radicals and injure tissues. MPO activity, which is an enzyme marker of leukocytes, has been used for estimation of the number of leukocytes migrated.

In our research, in order to estimate the number of leukocytes, MPO activity in gastric mucosa treated with increasing concentrations of ethanol was measured (Fig. 2). A significant increase in MPO activity after ethanol treatment was found compared to the control value in non-treated rats. Furthermore, MPO activity increased as the gastric lesion surface increased. This result supports the idea that polymorphonuclear leukocytes have migrated into the inflammation zone and that they may be responsible for the production of hydroxyl radicals that damage mucosa. Additionally, in our experiments, concentration of ethanol strongly affected occurrence of ulcer and when the concentration of ethanol was increased, the lesion surface and MPO activity in the mucosa increased. These results differed from those of Mizui and Doteuchi (1983), who did not find significant differences between ethanol concentrations of 40%, 60% and 80%. This may be explained by the fact that they used a different species of rat (Sprague–Dawley rats), which was larger than ours (250–350 g), for their experiments and thus observed a threshold concentration that did not occur in smaller rats.

The experiment conducted on the duration of treatment (Fig. 3) showed that MPO activity increased strongly when using 60% ethanol for 60 min and that the activity remained the same for longer durations, even if the lesion

surface increased. This result suggests that the initial inflammation and migration of activated leukocytes into the mucosa occurred within 1 h. Moreover, the expansion of lesion surface after that time seems to result from free radicals produced by leukocytes that have already migrated.

To summarize results related to ethanol-induced ulcer, ethanol seems to injure the mucosa and initiate the migration of activated leukocytes. As a response to inflammation, leukocytes produce $H_2O_2$ and $O_2^-$ radicals that cause injury deeper in the mucosa.

### 4.3. Effects of chlorogenic acid and procyanidins on gastric ulcer

Chlorogenic acid is one of most common polyphenols to occur, not only in pears but also in many fruits and vegetables, including apples, apricots, peaches, plums, raspberries, blueberries, avocado, carrots and eggplants (Shahidi & Naczk, 1995). Because this compound has antioxidant activity, we expected that chlorogenic acid might show some preventive effect on the formation of gastric ulcer. However, chlorogenic acid had no significant effect nor did it have a negative effect. The result of MPO activity indicated that chlorogenic acid administration, followed by 60% ethanol treatment, seemed to stimulate the migration of leukocytes more strongly than in the controls. From this, it could be considered that the mechanism of the promotive effect of chlorogenic acid on gastric ulcer would be stimulation of migration of leukocytes that produce reactive oxygen. In contrast to our result, some reports indicated that chlorogenic acid might have a preventive effect on gastric ulcer. Fujikata, Yamaguchi, Morita, Takeda, and Nishibe (1996) reported a significant inhibitory effect of chlorogenic acid on gastric ulcer in stressed rats restrained in cold water. Moreover, Graziani et al. (2005) reported that catechin or chlorogenic acid, the main phenolic components of apple extracts, were equally effective as apple extracts in preventing oxidative injury to human gastric epithelial cells. However, they used different procedures to induce damage in the model systems so that the mechanism of action of chlorogenic acid should differ from that shown by our results. Results of our research suggest that chlorogenic acid might promote formation of gastric lesion by stimulation of leukocyte migration when HCl/ethanol was used as an inducer. It is also important to note that, whereas an enzymatic antioxidant, such as SOD or catalase, is efficient against ulcer formation, a non-enzymatic antioxidant, such as catechin (Saito et al., 1998) or α-tocopherol (Osakabe et al., 1998) would have no significant effect on ethanol-induced gastric lesion. It is also remarkable that catechin appeared to have no effect on ethanol-induced gastric lesion (Saito et al., 1998) but was significantly efficient against stress-induced ulcer through water immersion restraint (Sato, Matsui, & Arakawa, 2002).

Procyanidins are widely distributed in the plant kingdom as the most abundant of the proanthocyanidins, which are polymers of a variable number of flavan-3-ol (catechins) units. Saito et al. (1998) studied the antiulcer capacity of pure procyanidin oligomers and showed that the antiulcer activity of a series of procyanidins increased as the degree of polymerization of the catechin unit increased. Oligomers longer than three catechin units showed a strong protective effect against stomach mucosal injury. They also reported that procyanidins, such as pentamers and hexamers, strongly bound to BSA. In our experiment, we observed that procyanidins covered the surface of the stomach (Fig. 5). Our data in Table 1 show that procyanidins extracted from WND had a very high mDP, indicating that these compounds may effectually bind to the mucosa. Thus, the mechanism of protection of the mucosa by pear procyanidins may be both physical and chemical. By binding strongly to the mucosa, procyanidins build a protective layer against ethanol, reducing leukocyte migration, and then deploying a local antioxidant protection against free radicals. The real chemical pathway for activation and migration of leukocytes is not well understood, and it is difficult to say at which level procyanidins prevent this migration. From our results (Table 2), at least 10 mg of pear procyanidins was sufficient to prevent ulcer and the possible negative effect of chlorogenic acid at the same dose. The results might also indicate that excessively purified compounds may have some dangerous aspects under some particular conditions, even those compounds that are known as health-promotive functional components.

In conclusion, procyanidins extracted from pear ('Winter Nélis') fruit have a high degree of antiulcer capacity, whereas chlorogenic acid alone seems to have a negative effect on HCl/ethanol-induced gastric ulcer. The mixture of those polyphenolics still showed a significant protective effect. Procyanidins appeared to bind strongly with the proteins of the mucosa and seemed to display a local protective effect against free radicals produced by activated leukocytes.

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