Antiulcerative Properties of Crude Polyphenols and Juice of Apple, and Chinese Quince Extracts

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Abbreviations: MPO, myeloperoxidase; UI, ulcer index.

Abstract

Effects of Chinese quince extract, apple juice, semi-purified phenolics and soluble pectin from these fruits on ethanol-induced gastric ulcers in rats were investigated. In rats given Chinese quince extract or apple juice, ulcer induction was strongly suppressed, and the effect was stronger for Chinese quince extract than for apple juice. Myeloperoxidase activity in gastric mucosa showed a similar tendency. The DPPH radical scavenging activity and total phenolic content were 4 times higher in Chinese quince extract than in apple juice. Semi-purified phenolics from both fruits strongly suppressed ulcer induction at doses of 5–10 mg; however, a 20-mg dose of apple phenolics showed a pro-ulcerative effect. The soluble pectin fraction also showed moderate activity. These results suggest that phenolic compounds are responsible for antiulcerative activity of Chinese quince extract and apple juice, and that concentration may be an important factor in the case of apple phenolics.

Keywords: polyphenols, procyanidin, chlorogenic acid, pectin, myeloperoxidase, gastric mucosa injury

1. Introduction

Phenolics, because of their strong antioxidant capacity along with anti-inflammatory, anticarcinogenic and antiallergic effects, are regarded as one of the functional compounds that contribute to the health-improving effects of various fruits, vegetables, and their derivatives (Wise, 2001; O'Neill, Standage, Hughes & Murray, 2001; Waladkhani & Clements, 2001). Recent studies have shown that some phenolics are absorbed from the digestive tract and act as health-promoting factors for the circulatory system (Manach, Scalbert, Morand, Rémésy & Jiménez, 2004); however, because the bioavailability of phenolics is relatively low (especially for higher molecular compounds), their action is mainly restricted to the digestive tract (Halliwell, Zhao & Whiteman, 2000).

Some phenolics have been reported to have antiulcerative properties in rats (Saito, Hosoyama, Ariga, Kataoka & Yamaji, 1998; Osakabe, Sanbongi, Yamagishi, Takizawa & Osawa, 1998; Galati et al., 2003). In our previous research, a procyanidin-rich fraction from fruits such as Chinese quince (Hamauzu, Inno, Kume, Irie & Hiramatsu, 2006) or pear (Hamauzu, Forest, Hiramatsu & Sugimoto, 2007) showed a strong preventive effect on gastric ulcers induced using HCl/ethanol in rats. However, in these reports we also showed that the chlorogenic acid standard or phenolic fraction from apples rich in chlorogenic acid showed a tendency to enhance HCl/ethanol-induced ulcers. Therefore, it remained to be clarified whether apple phenolics are harmful to this type of ulcer in all circumstances and whether Chinese quince phenolics are effective in preventing the ulcer only at normal consumption levels. Moreover, the effect of other accompanying components in these fruits should also be taken into account.

Dietary fibre, such as that found in pectic polysaccharides, is an example of a component accompanying phenolics in the edible part of fruits or fruit extracts. Dietary fibre is also regarded as an important functional component of fruits for human health because it has been associated with a lower risk of several gastrointestinal diseases (Ramakrishna, 2001); moreover, the pectic polysaccharides (pectin) have also been reported to have a number of pharmacological actions, such as hypoglycemic, cholesterol-decreasing, and antiulcerative activity (Wang, Pagán & Shi, 2002).

Phenolics and pectins present in fruits and their extracts are potential antiulcerative factors; hence it is possible that apple juice may prevent HCl/ethanol-induced ulcers. Moreover, it is interesting to compare the effect of these two components on gastric ulcer prevention, because only few experimental studies comparing their function have been reported, although both phenolics and pectins are relatively abundant in some fruits. The primary aim of the present study was to evaluate whether a commercially available Chinese quince extract or apple juice has a preventive effect on HCl/ethanol-induced ulcers. The secondary aim was to investigate the effect of dosage of fruit phenolics or pectins on inducing ulcer.

2. Materials and Methods

2.1. Food materials

Chinese quince fruit extract and apple juice (cloudy type) were purchased from a local market affiliated to a juice factory in Nagano prefecture, Japan. The Chinese quince extract was made using osmotic effect caused by the addition of sucrose and contained 60% (w/w) of the juice (pH 3.4). The apple juice was made from 'Fuji' apples and contained >12% Brix and 0.25% organic acid. The ripe fruits of Chinese quince and apple were obtained from a local orchard managed by the president of the market. 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, the samples were ground to powdered form using a mixer and stored in a desiccator for further use.

2.2. Solvents and reagents

(–)-Epicatechin was purchased from Sigma–Aldrich Ltd. (St. Louis, MO). Phlorizin was purchased from MP Biomedicals Inc. (Illkirch, France). Caffeic acid, (+)-catechin, chlorogenic acid standards and 3,5-dimethylphenol were purchased from Nacalai Tesque Inc. (Kyoto, Japan). α-D-Galacturonic acid was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Solvents were purchased from Nacalai Tesque, and TMB (3,3',5,5'-tetra-methyl-benzidine) from Moss Inc. (Pasadena, MD). Hydrogen peroxide solution (30%) 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). Apple pectin (P8471) was purchased from Sigma–Aldrich Ltd.

2.3. Preparation of fruit phenolic fraction

Before the extraction of phenolics, the freeze-dried flesh powder (10 g) was mixed with petroleum ether in a beaker, stirred and filtered through filter paper on a Büchner funnel to remove lipids (100 mL × 5 times). The phenolics were then extracted from the residue with 60% (v/v) aqueous acetone (100 mL × 2 times) in the same manner. The 60% acetone solution was evaporated using a rotary evaporator until all the organic solvent was removed. The aqueous solution of the extracts was applied onto a Sep-Pak Vac 20 cc (5 g) C18 cartridge column (Waters Co., Milford, MA), which was preconditioned with 10 mL of methanol and 0.1% (v/v) trifluoroacetic acid (TFA) in water. The column was washed with 40 mL of 0.1% TFA solution, and phenolics were eluted with 20 mL of methanol. The methanol solution was added to water and rotary evaporated, and the resultant aqueous solution was frozen and then freeze-dried to obtain semi-purified phenolic powder. The total phenolic content of the semi-purified powder was estimated by the Folin–Ciocalteu method using Folin–Ciocalteu reagent as described below. The phenolic powder obtained was also analyzed using HPLC for evaluation of phenolic composition.

2.4. Determination of total phenolics

The experimental procedure was adapted from that of Hamauzu, Yasui, Inno, Kume and Omanyuda (2005). The sample solution was mixed with Folin–Ciocalteu reagent, 2 mL each, in a test tube. After 3 min of reaction, 2 mL of Na_2CO_3 (10 g/100 mL) was added, and the mixture was incubated for 60 min at room temperature. 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 phenolics

Chromatographic separation was carried out on a Luna 5 μ C18 column (150 × 4.6 mm, Phenomenex Inc., Torrance, CA) at 40°C using two solvents: 0.1% TFA (solvent A) and 0.1% TFA in acetonitrile (solvent B). The gradient program was started with 5% B and changed to obtain 15%, 32%, 40% and 75% of B at 30, 35, 45 and 50 min, respectively. For the next 65 min, 75% B was maintained. 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 polyphenols was achieved by comparing retention times and UV spectra with those of standards. Peaks of oligomeric and polymeric procyanidins were calculated as (–)-epicatechin, for convenience. Thioacidolysis was also used to obtain information for identifying peaks.

2.6. Determination of average degree of polymerization of procyanidins

The average degree of polymerization of procyanidins 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 by Guyot, Marnet, Laraba, Sanoner and Drilleau (1998). The experimental procedures were adapted from those of Hamauzu et al. (2005). The standard curve for (–)-epicatechin benzylthioether was obtained by thioacidolysis of the procyanidin B_2 standard.

2.7. Preparation of soluble pectin fraction

The soluble pectin fraction from Chinese quince fruit was prepared by extraction with water from alcohol-insoluble solids (AIS) of the fruit. The preparation of AIS was as follows: freeze-dried flesh (75 g) was boiled in 500 mL of 80% (v/v) ethanol for 15 min and then passed through a filter paper on a Büchner funnel. The residue was washed with 80% (v/v) ethanol (500 mL) and then with 200 mL acetone to remove procyanidins. Diethylether (250 mL) was used to decolorize the residue. The decolorized residue was left at room temperature until diethylether was removed and then placed in a freeze-dryer overnight. The AIS obtained (27.5 g) was mixed to a duplicate and approximately 55 g was added to 1000 mL of water in a beaker and stirred overnight at room

temperature. The supernatant was then collected and centrifuged (8000*g*, 10 min, 4°C). The precipitate was re-suspended in the same volume of water, stirred and separated from the supernatant, which was combined, rotary evaporated to reduce the volume and then freeze-dried to produce dried material of soluble dietary fibre (soluble pectin fraction). The uronic acid content in the fraction was determined spectrophotometrically using galacturonic acid as a standard (described in 2.8).

2.8. Determination of pectin content

The soluble pectin content in the Chinese quince extract or apple juice was determined using a method described by Scott (1979), with the following modifications: to remove phenolics and neutral sugars, an aliquot (16 mL) of sample solution was added to acetone (24 mL), stirred vigorously, left for 10 min and then centrifuged (10000*g*, 20 min, 4°C). This treatment was repeated 4 times, and the sample was then washed with ethanol. The precipitate was dissolved in 10 mL of water, and the uronic acid content of the aqueous solution was determined spectrophotometrically as follows: An aliquot (0.25 mL) of test solution was added to the same volume (0.25 mL) of 2% (w/v) sodium chloride solution in a test tube. Concentrated sulphuric acid (4 mL) was added to the test tube in an ice bath and then heated for 10 min at 70°C. After the reactant cooled to room temperature, 0.1% (w/v) 3,5-dimethylphenol (in glacial acetic acid) (0.2 mL) was added to the reactant and mixed. After 10 min at room temperature, absorbance at 450 and 400 nm was measured. A blank made in the same manner, except that glacial acetic acid was added instead of 3,5-dimethylphenol reagent. ΔA ($A_{450}-A_{400}$) was used to measure absorbance for uronic acid to calculate the content. Total uronic acid content (pectin content) was calculated using α-D-galacturonic acid as the standard.

2.9. DPPH radical scavenging activity

The DPPH radical scavenging activity of Chinese quince extract and apple juice was expressed as the EC_{50} value, defined as the volume of the sample that could scavenge 50% of DPPH in the experimental system. The experimental procedure was the same as that described in our previous report (Hamauzu et al., 2006).

2.10. Antiulcer test

2.10.1. Animals

Male Wistar rats (Jcl: Wistar) weighing 230–262 g were obtained from CLEA Japan Inc. (Tokyo, Japan), 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 days. The animals were fasted for 24 h before the experiment and allowed free access to water. The experiments were approved by the ethics committee of Shinshu University.

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186 2.10.2. Treatment

187 Each rat was given 3 mL of Chinese quince extract or apple juice intragastrically and then given
188 1.5 mL of acidified ethanol solution (150 mM HCl/ethanol = 40:60 v/v) after 30 min. The control rats
189 were given 3 mL of water instead of the test solution. In case of the extracted phenolic or pectin
190 fraction, 5–20 mg of the substance was suspended in 1.5 mL of water and then given to rats. The
191 animals were sacrificed under anesthesia 60 min after HCl/ethanol administration. Their stomachs
192 were removed, opened along the greater curvature and rinsed with physiological saline. The rat
193 stomachs were then stretched on balsa boards and pinned with the mucosal side up. Digital pictures
194 of the mucosal surface of each stomach were taken for morphometrical analysis, as described below,
195 and the stomachs were cut along the lesser curvature with razor blades and divided into half. One
196 half was frozen with liquid nitrogen and kept at –20°C under nitrogen gas for further evaluation of
197 myeloperoxidase activity in the mucosa. The other half was processed for histological analysis.

198

199 2.10.3. Analysis of lesions

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

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205 2.11. Measurement of myeloperoxidase (MPO) activity

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207 A crude enzyme solution was prepared from homogenized mucosa randomly collected with a
208 razor blade from the inner surface of the frozen stomach. MPO activity was measured
209 spectrophotometrically using TMB and 0.3% H_2O_2 in acetate buffer (pH 5). The experimental
210 conditions were the same as those described in our previous report (Hamauzu et al., 2007).

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212 2.12. Statistical analysis

213 Results are expressed as mean ± SE. Means were compared with the Turkey–Kramer test using
214 Excel 2002 with the add-in software Statcel 2 (OMS, Tokyo, Japan). Differences were considered
215 significant at $P < 0.05$.

216

217 **3. Results and Discussion**

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219 3.1. Antiulcerative effect, DPPH scavenging activity, soluble pectin content and phenolic profile of
220 Chinese quince extract and apple juice

221

222 Chinese quince extract and apple juice, both showed a strong preventive effect on gastric ulcers
223 induced by HCl/ethanol. By macroscopic observation, the acute ulcer induced by HCl/ethanol

appeared to have intense gastric hyperemia extending in a band-like conformation and consisting of thickened lesions as well as many filiform lesions. These signs were observed to a marked degree in control rats that were given only water before inducing ulcer by the administration of HCl/ethanol (Fig. 1B). In contrast, gastric ulcer induction was strongly suppressed in rats that were given Chinese quince extracts or apple juice, and the effect was stronger in those given Chinese quince extract (Fig. 1C and D). The intensity of the gastric ulcer, as quantified by the percentage of the injury area, was 20% in control rats versus 0.002% and 2.1% in rats given Chinese quince extract and apple juice, respectively (Table 1). MPO activity in gastric mucosa (22.3 U/mg protein in controls) also was suppressed significantly ($P < 0.05$) in rats given Chinese quince extract (10.5 U/mg protein) and tended to be suppressed in rats given apple juice (11.6 U/mg protein) as well. The free radical scavenging activity of Chinese quince extract, expressed as the volume (mL) that can scavenge 50% of DPPH, was 4 times stronger than that of apple juice.

From these results, it appeared that the preventive effect of Chinese quince extract or apple juice might be due to the radical scavenging capacity and the suppression of leukocyte migration to the gastric mucosa, which could be indicated by lowered activity of MPO, a marker enzyme of leukocytes. It has been thought that leukocytes migrate to the site of inflamed mucosa after injury by HCl/ethanol and subsequently expand the lesion area by producing active oxygen species, including free radicals (Osakabe et al., 1998). Therefore, suppression of leukocyte migration may be an important mechanism of action in the antiulcerative activity as well as radical scavenging capacity of the fruit extract and juice.

Results of pectin and phenolic analysis showed that Chinese quince extract contained 1.3 mg/100 mL of soluble pectin and 324 mg/100 mL of total phenolics (Table 1). In comparison, apple juice contained 4.9 mg/100 mL of soluble pectin and 85 mg/100 mL of total phenolics. Furthermore, the phenolic profiles of Chinese quince extract and apple juice analyzed by RP-HPLC were quite different. Phenolics of Chinese quince extract were mainly composed of (–)-epicatechin and its polymerized compounds (polymeric and oligomeric procyanidins) and relatively small amounts of caffeoylquinic derivatives. In contrast, phenolics of apple juice were mainly composed of 5-caffeoylquinic acid, (–)-epicatechin, procyanidin B2 and two phloretin derivatives.

These results suggested that the phenolic compounds were the major factor influencing radical scavenging capacity, because there was a relationship between the phenolic concentration and the DPPH radical scavenging activity; both the phenolic concentration and the DPPH radical scavenging activity were 4 times greater in Chinese quince extract than in apple juice. The findings also suggest that the effect of compositional differences in phenolics between Chinese quince extract and apple juice on DPPH radical scavenging activity was small. Therefore, the advantage in antiulcerative action of phenolics in Chinese quince extract might be due to the high concentration of procyanidins and their binding ability to mucosal tissue (Saito et al., 1988), which could help in maintaining the antioxidant action in the gastric wall.

It is also worth noting that apple juice showed significant antiulcerative activity despite a potential pro-ulcerative effect of its phenolic composition (Hamauzu et al., 2006). In fact, in our

previous study, we observed that apple phenolics and chlorogenic acid standard tended to promote the HCl/ethanolinduced ulcer (Hamauzu et al., 2006, 2007). For that reason, it should be clarified whether the antiulcerative activity of apple juice observed in current experiment was due to the concentration of phenolics or existence of another antiulcerative component, such as pectin. Pectin has been reported to have a preventive effect on gastric ulcers (Sun, Matsumoto & Yamada, 1992; Dunjic, et al., 1993; Wang, Pagán & Shi, 2002); therefore, it was expected to be another antiulcerative factor in apple juice or even the Chinese quince extract. For this reason, we tested the antiulcerative activity of semi-purified phenolics from both fruits and extracted soluble pectins from Chinese quince fruit and apple in different dosages.

3.2. Composition of semi-purified phenolics

The semi-purified phenolics from Chinese quince and apple fruits were analyzed by RP-HPLC to check their compositional differences. Chinese quince phenolics contained 73.5% of oligomeric and polymeric procyanidins and 0.65% of caffeoylquinic derivatives as characteristic components (Table 2). In apple phenolics, 5-caffeoylquinic acid (22.1%), catechins (13.7%), dimeric and oligomeric procyanidins (18.1%), and phloretin derivatives (4.1%) were the main components. The average degree of polymerization of procyanidins was 18.6 and 3.0 for Chinese quince and apple phenolics, respectively.

Few compositional differences were noted between phenolics semi-purified from fruits and that in Chinese quince extract or apple juice. However, the percentage of procyanidins in the semi-purified phenolics was higher than that in the beverages (extract or juice). Because the degree of polymerization of procyanidins and the amount of these substances in the fruits were quite different, the compositional difference of the fruit phenolics was more remarkable than those between the beverages.

3.3. Effect of semi-purified phenolics and soluble pectin from Chinese quince and apple fruits on the gastric ulcer

Semi-purified phenolics from Chinese quince and apple fruits were administered to rats in doses of 0 (control), 5, 10 and 20 mg to investigate the effect of dosage on gastric ulceration. In rats that were given Chinese quince phenolics, the area of gastric lesion was significantly smaller (0.7–3.3%) compared with controls (20.2%), and the effect was dose dependent (Fig. 2A). In rats that were given the apple phenolics, the lesion area was also significantly smaller (4.2–6.4%) in those given the 5- or 10-mg dose; however, this result was not dose-dependent. Moreover, in rats given 20 mg of apple phenolics, the lesion area was significantly larger (34.5%) than in controls. MPO activity in mucosa of rats given 5 mg of Chinese quince phenolics was significantly higher (40.5 U/mg protein) than that of controls (22.3 U/mg protein), and activity decreased with an increase in dosage (Fig. 2B). A similar tendency was observed in rats given apple phenolics.

Thus, the effect of crude phenolics from Chinese quince and apple fruits on the HCl/ethanol-induced ulcer differed: the former showed dose-dependent ulcer prevention and the latter did not. However, it is noteworthy that apple phenolics administered at a dose of 5 or 10 mg showed a significant antiulcerative effect, although they also showed a significant pro-ulcerative effect at a dose of 20 mg. The result indicates that apple phenolics may be effective in preventing ulcers at a low dosage. In the case of the experiment with apple juice, the phenolics given to rats were approximately 2.6 mg/3 mL dose. This might be an effective dose of apple phenolics to produce the antiulcerative activity. It should be emphasized that the natural concentration of phenolics in both apple fruit and juice may not cause any deteriorating effect on HCl/ethanol-induced gastric ulcers and, in fact, may have a healthy benefit. Moreover, at the 5-mg dose, the antiulcerative effect of apple phenolics and that of Chinese quince phenolics was almost equivalent. Therefore, it can be concluded that when ingested at a natural (realistic) concentration, apple phenolics were not inferior to Chinese quince phenolics as an active compound for HCl/ethanol-induced ulcers. However, an excess dose of purified compounds seemed to increase the risk of adverse effects, even if they are recognized as functional compounds; thus, care should be taken when concentrated extracts are used as supplements. This caution may also hold true for Chinese quince phenolics.

The strong antiulcerative activity of Chinese quince phenolics with a dose-dependent effect must be due to the presence of high amount of procyanidins, especially highly polymerized molecules. The proportion of procyanidins in total phenolics of fruit has been shown to be an important factor in antiulcerative activity, because quince phenolics, which contain a smaller proportion of procyanidins than Chinese quince phenolics, showed only moderate antiulcerative activity (Hamauzu et al., 2006). Moreover, the procyanidin fraction from pear fruit showed quite strong activity with high affinity for mucosal tissue (Hamauzu et al., 2007). It has been shown that procyanidin oligomers with a higher degree of polymerization demonstrate greater ability to bind to BSA (Saito et al., 1998), and it may be that these substances bind to the surface of mucosal tissue and act as a protective coating having a radical scavenging activity.

Concerning the harmful aspects of a high dosage of apple phenolics, administration of 20 mg of chlorogenic acid tended to expand the area of the gastric lesion (Hamauzu et al., 2007); thus, it was conceivable that the chlorogenic acid-rich phenolic fraction showed pro-ulcerative activity when administered at an excessive dose. However, Okada, Kudoh, Fukushi, Onodera, Kawabata and Shiomi (2005) reported that chlorogenic acid (100–200 mg/kg bw) effectively protected against gastric mucosal damage induced by ethanol in rats. Their experimental procedure was slightly different from ours; they used 5% aqueous solution of gum arabic to suspend the test compounds and used 99.5% ethanol for the ulcer induction. This raises the possibility that the action of chlorogenic acid might be affected by environmental conditions in the gut. The mechanism of action for the pro-ulcerative effect of apple phenolics observed in our study seemed to differ from the stimulation of leukocyte migration because MPO activity was lower at a higher dosage (Fig. 2B). Additionally, at a lower dose, administration of both fruit phenolics seemed to stimulate leukocyte migration more strongly than was noted in controls. It seemed that the antioxidant property of phenolics affected the

ability to scavenge the reactive oxygen species generated by leukocytes and prevented expansion of the lesion area, except in the case of the 20-mg dose of apple phenolics. Further investigation of the action in the case of excessive doses of apple phenolics or chlorogenic acid is required. In any case, at a realistic dosage level, these phenolics may exhibit a beneficial effect in the prevention of gastric ulcers related to the generation of reactive oxygen species (Graziani et al., 2005).

Administration of soluble pectin fraction from Chinese quince or apple fruit (containing 37.3% and 62.8% of galacturonic acid, respectively) also tended to suppress ulcer induction. The area of gastric lesion in rats given 5 and 10 mg of Chinese quince pectin fraction was 8.3% and 7.8%, respectively (Table 3). The area of lesion in rats given the same dose of apple pectin was similar to that in rats given Chinese quince pectin fraction (9.0% and 7.6%, respectively). MPO activity in the mucosa of rats given soluble pectin fraction from these fruits was nearly the same as that of controls, and no differences were noted between the fruits or the dosages.

These results suggested that in addition to phenolics, pectin in Chinese quince or apple fruit may function as an antiulcerative factor. Several researchers have reported that pectic polysaccharides may be responsible for antiulcerative activity (Yamada, 1994; Galati, Pergolizzi, Miceli, Monforte & Tripodo, 2002; Nergard et al., 2005). One mechanism proposed to explain this activity is the binding of pectic polysaccharides to the surface mucosa, which is thought to produce a protective coating (Yamada, 1994; Nergard et al., 2005). This proposed mechanism may be supported by recent research showing that pectin-like galacturonides can adhere strongly to mucous membranes in the colon (Schmidgall & Hensel, 2002). Therefore, it is possible that pectin in Chinese quince extract or apple juice partially contributed to the antiulcerative activity by forming a protective coating. However, the soluble pectin content in Chinese quince extract or apple juice was quite small compared with the phenolic content (Table 1); soluble pectin concentration of Chinese quince extract was only 0.4% of the phenolic concentration and that of apple juice was 5.8%. Moreover, even at the same dose (5 or 10 mg), the pectin fraction from Chinese quince or apple fruit showed a relatively smaller effect on gastric ulcer prevention than did phenolics (Fig. 2A and Table 3). Therefore, pectin might not be a major factor for antiulcerative activity of Chinese quince extract and apple juice in current concentrations, although pectin might have some additional effect to benefit health benefit.

4. Conclusion

The present study showed that the main functional factor for antiulcerative activity of Chinese quince extract and apple juice is phenolic compounds, although pectin may have contributed an additional effect on this activity or other health benefits. The principal mechanism of action appears to be the radical scavenging activity of both phenolics; in Chinese quince phenolics, the formation of a protective mucosal coating and maintenance of the radical scavenging effect might be additional mechanisms. The appropriate concentration of phenolics is an important factor for the expression of antiulcerative activity by apple phenolics in cases of HCl/ethanol-induced ulcers. The antiulcerative

effect tended to be stronger with lower dosage of apple phenolics, and the natural concentration of phenolics in apple fruit or juice seemed to be appropriate for providing beneficial health effects in the gastrointestinal tract.

References

Dunjic, B. S., Svensson, I., Axelson, J., Adlercreutz, P., Ar'Rajab, A., Larsson, K., & Bengmark, S. (1993). Green banana protection of gastric mucosa against experimentally induced injuries in rats. A multicomponent mechanism? *Scandinavian Journal of Gastroenterology*, *28*, 894–898.

Galati, E. M., Mondello, M. R., Giuffrida, D., Dugo, G., Miceli, N., Pergolizzi, S., & Taviano, M. F. (2003). Chemical characterization and biological effects of Sicilian *Puntia ficus indica* (L.) mill. Fruit juice: antioxidant and antiulcerogenic activity. *Journal of Agricultural and Food Chemistry, 51*, 4903–4908.

Galati, E. M., Pergolizzi, S., Miceli, N., Monforte, M. T., & Tripodo, M. M. (2002). Study on the increment of the production of gastric mucus in rats treated with Opuntia ficus indica (L.) Mill. Cladodes. *Journal of Ethnopharmacology*, *83*, 229–233.

Graziani, G., D'Argenio, G, Tuccillo, C., Loguercio, C., Ritieni, A., Morisco, F., Del Vecchio Blanco, C., Fogliano, V., & Romano, M. (2005). Apple polyphenol extracts prevent damage to human gastric epithelial cells in vitro and to rat gastric mucosa in vivo. *Gut, 54*, 193–200.

Guyot, S., Marnet, N., Laraba, D., Sanoner, P., & Drilleau, J.-F. (1998). Reversed-phase HPLC following thiolysis for quantitative estimation and characterization of the four main classes of phenolic compounds in different tissue zones of a french cider apple variety (*Malus domestica* var. Kermerrien). *Journal of Agricultural and Food Chemistry, 46*, 1698–1705.

Halliwell, B., Zhao, K., & Whiteman, M. (2000). The gastrointestinal tract: A major site of antioxidant action? *Free Radical Research*, *33*, 819–830.

Hamauzu, Y., Forest, F., Hiramatsu, K., & Sugimoto, M. (2007). Effect of pear (*Pyrus communis* L.) procyanidins on gastric lesions induced by HCl/ethanol in rats. *Food Chemistry*, *100*, 255–263.

Hamauzu, Y., Inno, T., Kume, C., Irie, M., & Hiramatsu, K. (2006). Antioxidant and antiulcerative properties of phenolics from Chinese quince, quince, and apple fruits. *Journal of Agricultural and Food Chemistry, 54*, 765–772.

Hamauzu, Y., Yasui, H., Inno, T., Kume, C., & Omanyuda, M. (2005). Phenolic profile, antioxidant property, and anti-influenza viral activity of Chinese quince (*Pseudocydonia sinensis* Schneid.), quince (*Cydonia oblonga* Mill.), and apple (*Malus domestica* Mill.) fruits. *Journal of Agricultural and Food Chemistry, 53*, 928–934.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*, *79*, 727–747.

Nergard, C. S., Diallo, D., Inngjerdingen, K., Michaelsen, T. E., Matsumoto, T., Kiyohara, H., Yamada, H., & Paulsen, B. S. (2005). Medicinal use of *Cochlospermum tinctorium* in Mali. Anti-ulcer-, radical scavenging- and immunomodulating activities of polymers in the aqueous extract of the roots. *Journal of Ethnopharmacology*, *96*, 255–269.

O'Neill, K. L., Standage, S. W., Hughes, B. G., & Murray, B. K. (2001). Fruits and vegetables and the prevention of oxidative DNA damage. In R. R. Watson, *Vegetables, fruits, and herbs in health promotion* (pp. 135–146). Boca Raton: CRC Press.

Okada, H., Kudoh, K., Fukushi, E., Onodera, S., Kawabata, J., & Shiomi, N. (2005). Antioxidative activity and protective effect of fermented plant extract on ethanol-induced damage to rat gastric mucosa (In Japanese with English summary). *Journal of Japanese Society of Nutrition and Food Science*, *58*, 209–215.

Osakabe, N., Sanbongi, C., Yamagishi, M., Takizawa, T., & Osawa, T. (1998). Effects of polyphenol substances derived from *Theobroma cacao* on gastric mucosal lesion induced by ethanol. *Bioscience, Biotechnology and Biochemistry, 62*, 1535–1538.

Ramakrishna, B. S. (2001). Gastrointestinal nutritional problems in the aged: Role of vegetable and fruit use. In R. R. Watson, *Vegetables, fruits, and herbs in health promotion* (pp. 233–255). Boca Raton: CRC Press.

Saito, M., Hosoyama, H., Ariga, T., Kataoka, S., & Yamaji, N. (1998). Antiulcer activity of grape seed extract and procyanidins. *Journal of Agricultural and Food Chemistry, 46*, 1460–1464.

Schmidgall, J., & Hensel, A. (2002). Bioadhesive properties of polygalacturonides against colonic epitherial membranes. *International Journal of Biological Macromolecules*, *30*, 217–225.

Scott, R. W. (1979). Colorimetric determination of hexuronic acids in plant materials. *Analytical Chemistry*, *51*, 936–941.

Sun, X. B., Matsumoto, T., & Yamada, H. (1992). Purification of an anti-ulcer polysaccharide from the leaves of Panax ginseng. *Planta Medica*, *58*, 445–448.

Waladkhani, A. R., & Clemens, M. R. (2001). Effect of dietary phytochemicals on cancer development. In R. R. Watson, *Vegetables, fruits, and herbs in health promotion* (pp. 3–18). Boca Raton: CRC

Press.

Wang, Q., Pagán, J., & Shi, J. (2002). Pectin from fruits. In J. Shi, G. Mazza, & M. Le Maguer, *Functional foods*, vol. 2 (pp. 263–309). Boca Raton: CRC Press.

Wise, J. A. (2001). Health benefits of fruits and vegetables: The protective role of phytonutrients. In R. R. Watson, *Vegetables, fruits, and herbs in health promotion* (pp. 147–176). Boca Raton: CRC Press.

Yamada, H. (1994). Pectic polysaccharides from Chinese herbs: structure and biological activity. *Carbohydrate Polymers*, *25*, 269-276.

Figure Captions.

Fig. 1. Photographs showing inner surface of rat stomach. (A) No treatment (normal stomach of rat); (B) Control (water administered before treatment with 60% ethanol containing 1.5 mM HCl); (C) Rat administered Chinese quince extract before treatment; (D) Rat administered apple juice before treatment.

Fig. 2. Intensity of gastric ulcer (A) and myeloperoxidase activity of mucosa (B) of rats that were administered Chinese quince polyphenols (CQPP) or apple polyphenols (APP) before treatment with 60% ethanol containing 1.5 mM HCl. Error bars indicate standard error (n = 15 for control rats administered water containing no polyphenols; n = 5 for each group administered each amount of fruit polyphenols).

Table 1
Antiulcerative property, free radical scavenging activity, soluble pectin content, total phenolic content and phenolic composition of Chinese quince extract and apple juice

	Chinese quince extract	Apple juice
Antiulcerative effect		
Area of gastric legion (%) [a]	0.002 ± 0.002*	2.1 ± 0.4*
(% suppression [b])	(99.9)	(10.4)
MPO activity of mucosa (U/mg protein) [c]	10.5 ± 1.9*	11.6 ± 3.6
(% suppression [b])	(47.1)	(52.0)
Free radical scavenging activity (EC_{50}) [d]	0.03 ± 0.001	0.12 ± 0.01
Soluble pectin (mg/100 mL) [e]	1.3 ± 0.07	4.9 ± 0.2
Total phenolics (mg/100 mL) [f]	342.2 ± 21.5	85.0 ± 6.4
Phenolic composition [g]		
(+)-Catechin	nd	0.57 ± 0.07
(–)-Epicatechin	3.7 ± 0.6	3.1 ± 0.09
Procyanidin B1 [h]	2.3 ± 0.2	1.3 ± 0.03
Procyanidin B2 [h]	7.3 ± 1.9	4.1 ± 0.07
Oligomeric procyanidins [h]	11.9 ± 3.2	tr
Polymeric procyanidins [h]	106.1 ± 38.8	nd
3-Caffeoylquinic acid [i]	4.9 ± 0.7	nd
5-Caffeoylquinic acid	5.5 ± 0.5	17.0 ± 0.2
Phloretin derivative [j]	nd	0.86 ± 0.01
Phlorizin	nd	0.70 ± 0.01

Data are mean ± SE (n = 5 for antiulcerative assays; n = 3 for radical scavenging activity and analysis of components). * $P < 0.05$ vs control in antiulcerative assays. Abbreviations: nd, not detected; tr, trace.

[a] Percentage of legion area in total surface area of stomach. Rats were administered 3 mL of extract or juice before gastric ulcer induction. Value of control rats that were administered 3 mL of water was 20.2 ± 2.4%.

[b] (Value of rats administered each sample/Value of control rats) × 100.

[c] Value of control rats was 22.3 ± 2.7 U/mg protein.

[d] Values are volume (mL) of sample that can scavenge 50% of DPPH.

[e] Values are expressed as α-galacturonic acid equivalent.

[f] Values are expressed as (–)-epicatechin equivalent in Folin–Ciocalteu method.

[g] Values are results of HPLC analysis and expressed as mg/100 mL.

[h] Values were calculated using standard curve for (–)-epicatechin.

[i] Values were calculated using standard curve for 5-caffeoylquinic acid.

[j] Values were calculated using standard curve for phlorizin.

Table 2
Main phenolic components and average degree of polymerization of procyanidins in semi-purified phenolic fraction of Chinese quince and apple fruit

	Chinese quince	Apple
(+)-Catechin	nd	1.6%
(–)-Epicatechin	tr	12.1%
Procyanidin B1[a]	tr	1.1%
Procyanidin B2 [a]	tr	8.8%
Oligomeric and polymeric procyanidins [a]	73.5%	8.2%
3-Caffeoylquinic acid [b]	0.33%	nd
5-Caffeoylquinic acid	0.32%	22.1%
Phloretin derivative [c]	nd	1.9%
Phlorizin	nd	2.2%
mDP of procyanidins	18.6	3.0

Data are expressed as milligrams of each compound included in 100 mg of total phenolics assessed by Folin–Ciocalteu method.
Abbreviations: nd, not detected; tr, trace; mDP, number average degree of polymerization.
[a] Values are calculated using standard curve for (–)-epicatechin.
[b] Values are calculated using standard curve for 5-caffeoylquinic acid.
[c] Values are calculated using standard curve for phlorizin.

Table 3
Intensity of gastric ulcer and myeloperoxidase activity of mucosa of rats that were administered water (control), soluble pectin from Chinese quince or apple pectin before treatment with 60% ethanol containing 1.5 mM HCl

	Area of gastric legion (% of total area)	MPO activity of mucosa (U/mg protein)
Control	20.2 ± 2.4 a	22.3 ± 2.7 a
Chinese quince pectin		
5 mg	8.3 ± 2.2 ab	15.1 ± 3.3 a
10 mg	7.8 ± 3.9 b	16.5 ± 2.4 a
Apple pectin		
5 mg	9.0 ± 2.3 ab	17.7 ± 3.3 a
10 mg	7.6 ± 2.9 b	15.6 ± 5.5 a

Values are mean ± SE (n = 5). Means with the same letter are not significantly different ($P < 0.05$).

Fig 1. Hamauzu et al.

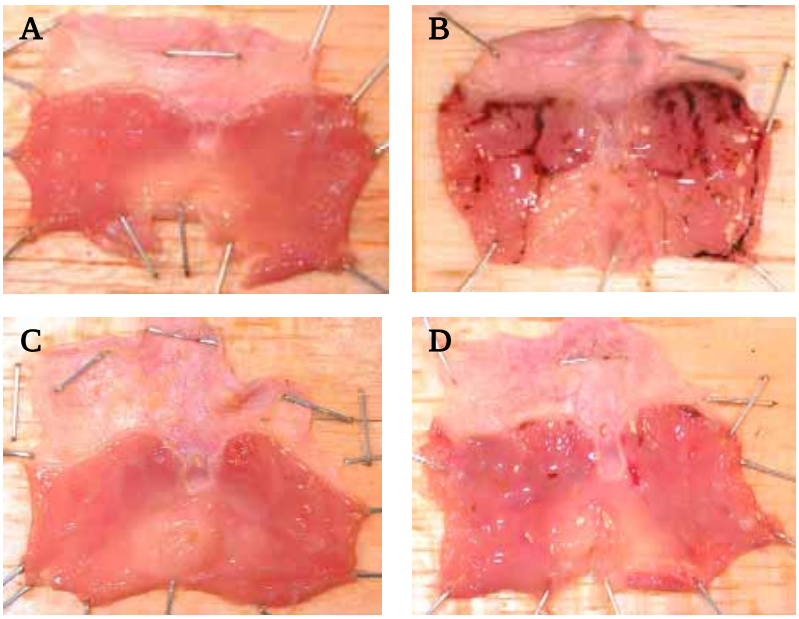

Fig 2. Hamauzu et al.