Anti-ulcerogenic Effect and HPLC Analysis of the Caffeoylquinic Acid-Rich Extract from *Ligularia stenocephala*

Byung-Il Lee,[a] Agung Nugroho,[b] Moch Saiful Bachri,[c] Jongwon Choi,[c] Kang Ro Lee,[d] Jae Sue Choi,[e] Won-Bae Kim,[f] Kyung-Tae Lee,[g] Jong-Dai Lee,[a] and Hee-Juhn Park*,[h]

[a] *Department of Food Science and Biotechnology, Kangwon National University; Chuncheon 200–701, Korea:* [b] *Department of Agro-industrial Technology, Faculty of Agriculture, Lambung Mangkurat University; Indonesia 70712, Indonesia:* [c] *College of Pharmacy, Kyungsung University; Busan 608–736, Korea:* [d] *Natural Products Laboratory, College of Pharmacy, SungKyunKwan University; Suwon 440–736, Korea:* [e] *Division of Food Science and Biotechnology, Pukyong National University; Busan 608–737, Korea:* [f] *Highland Agriculture Research Center, Rural Development Administration; Pyeongchang 232–950, Korea:* [g] *College of Pharmacy, Kyung-Hee Univerisity; Seoul 130–701, Korea: and* [h] *Department of Pharmaceutical Engineering, Sangji University; Wonju 220–702, Korea.*

Received August 11, 2009; accepted December 2, 2009

The leaves of three *Ligularia* species belonging to the family Compositae, *Ligularia stenocephala*, *L. fischeri*, and *L. fischeri* var. *spiciformis*, were qualitatively and quantitatively analyzed on the caffeoylquinic acids by HPLC and subjected to peroxynitrite-scavenging assay. The IC_{50} of the MeOH extract of *L. stenocephala* was 1.62±0.03 μg/ml and the major caffeoylquinic acids of *L. stenocephala* were 5-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoyl-*muco*-quinic acid, and 3,5-di-*O*-caffeoylquinic acid. The compositions of caffeoylquinic acids were different for the three plants. Since percentage of total caffeoylquinic acids of the extract was highest (42.20% of the MeOH extract and 94.52% of the BuOH extract) in *L. stenocephala* and potent in peroxynitrite-scavenging assay, the extracts of *L. stenocephala* were chosen to perform *in vivo* anti-ulcerogenic activity. Treatment of mice with the MeOH- and BuOH extracts decreased the diameter of gastric lesions caused by HCl/ethanol- and indomethacin/bethanechol and decreased the volume of gastric juice, suggesting that caffeoylquinic acids have anti-ulcerogenic activity. These results suggest that the leaves of *Ligularia* species may help prevent or treat gastric ulcers.

Key words *Ligularia stenocephala*; Compositae; caffeoylquinic acid; HPLC; peroxynitrite; ulcerogenic

Peptic ulcers can be caused by food, stress, genetic, and environmental factors,[1] including *Helicobacter pylori* infection, abuse of steroidal- or non-steroidal anti-inflammatory drugs (NSAIDs), irregular eating habits, smoking, alcohol consumption, and psychological stress.[2] Peptic ulcers result from defects in the mucosa and muscularis mucosa, as well as oxidative stress due to the increase of reactive oxygen species (ROS).[3]

In Korea, the leaves of *Ligularia stenocephala* Matsum. *et* Koidz. (Family Compositae) are consumed as an edible vegetable and used as a Chinese folk medicine for the treatment of edema and scrofula.[4] In eastern and western Asia, the genus *Ligularia* is used for the treatment of influenza, cough, ulcer, and tuberculosis.[5] Yoon *et al.* have reported that the leaf extract of *L. stenocephala* showed the highest antithrombotic activity among large numbers of edible and herbal plants.[6] The root of *L. stenocephala* contain benzofuran derivatives (ligulachepalins, euparin, hydroxytremetone,[7] 5,6-dimethoxy-2-isopropenylbenzofuran,[8] and stenocephalain,[9] as well as two caffeoylquinic acid derivatives, 3,4-dicaffeoylquinic acid and 3,5-dicaffeoylquinic acid).[6] However, caffeoylquinic acid levels of those plants have not been studied.

Although *L. stenocephala* has been traditionally used to treat gastric cancer or gastric ulcers, these biological activities have not been scientifically verified. Therefore, we intended to find the preventive activity of the caffeoylquinic acid-rich fraction with potent peroxynitrite scavenging effect on the ulcers induced by HCl/ethanol- and indomethacin/bethanechol-induced in mice. In addition, we attempted to find whether or not the wild vegetables such as *Ligularia* species containing high level of caffeoylquinic acids are beneficial for ulcer disease. The methanolic extract of *L. stenocephala* together with its BuOH- and Et_2O soluble fractions were evaluated for their anti-ulcerogenic activity using mice, with the BuOH extract showing the best activity. The BuOH fraction contained 5-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoyl-*muco*-quinic acid, and 3,5-di-*O*-caffeoylquinic acid from HPLC analytical data.

In Korea, three *Ligularia* species, *Ligularia stenocephala*, *L. fischeri*, and *L. fischeri* var. *spiciformis*, are used as edible vegetables. The three herbs are similar taxonomically and are called gondalbi, gomchwi and numchwi, respectively, in Korea. Distinguishing these species may be improved with HPLC chromatograms. Other Compositae family members also contain caffeoylquinic acids, including *Aster* species,[10] and *Solidago* species,[5,11] as well as *L. stenocephala*,[6] and *L. fischeri* var. *spiciformis*.[11]

Caffeoylquinic acid chemically consists of the two parts of quinic acid moiety and phenylpropanoid moiety. The most common parent skeleton of those compounds is quinic acid while *muco*-quinic acid or *epi*-quinic acid are also uncommonly found. The most common phenylpropanoid is caffeic acid while the less common ones are *p*-coumaric acid and ferulic acid. Caffeoylquinic acids are structurally classified into the monocaffeoylquinic acids and dicaffeoylquinic acids; however, tricaffeylquinic acids are very uncommon.

MATERIALS AND METHODS

Plant Materials The leaves of *L. stenocephala*, *L. fischeri*, and *L. fischeri* var. *spiciformis* (compositae) were col-

* To whom correspondence should be addressed. e-mail: hjpark@sangji.ac.kr

lected in the Alpine Agriculture Research Institute, Rural Development Administration, Korea, in June, 2008. Those leaves were dried and pulverized for extraction. These plants were identified by Dr. W. B. Kim (Alpine Agriculture Research Institute, Rural Development Administration, Korea). Voucher specimens were deposited in the laboratory of Natural Product Chemistry, Department of Pharmaceutical Engineering, Sangji University, Korea.

Extraction of Three *Ligularia* Species and HPLC Analysis To analyze caffeoylquinic acid content, plant material (20 g) together with MeOH (400 ml) were sonicated at 40 °C for 6 h in a 500 ml Erlenmeyer flask. The extract was filtered using filter paper and concentrated to dryness on a rotatory evaporator at 60 °C. Afterward, the extract was frozen (−80 °C) and dried on a freeze-dryer for 24 h to produce a powdery solid extract. For HPLC analysis, this extract was dissolved in 80% aqueous MeOH and filtered through a 0.50 μm syringe filter, then the filtrate (20 μl) was injected into the analyzer.

HPLC analysis was conducted on a Varian HPLC system (Walnut Creek, CA, U.S.A.) that includes a Prostar 210 solvent delivery module, a Prostar 325 UV–Vis detector, and a 20 μl sample loop (Rheodyne, Rohnert Park, CA, U.S.A.). Compound separation was performed on a Shiseido (Chuoku, Tokyo, Japan) Capcell Pak C18 column (5 μm, 250 mm× 4.6 mm i.d.). All solvents used for analysis were HPLC grade and obtained from J.T. Baker (Phillipsburg, NJ, U.S.A.). Seven standard caffeoylquinic acids isolated from *Lactuca indica* L.,[12,13] were offered from Prof. Kang Ro Lee (College of Pharmacy, SungKyunKwan University, Suwon, Korea). The seven caffeoylquinic acids are abbreviated as follows: 3,4-di-*O*-caffeoylquinic acid (3,4-DQ), 3,5-di-*O*-dicaffeoyl-*muco*-quinic acid (3,5-DmQ), 3,5-di-*O*-caffeoylquinic acid (3,5-DQ), 4,5-di-*O*-caffeoylquinic acid (4,5-DQ), 5-*O*-caffeoylquinic acid (5-CQ), 3-*O*-caffeoylquinic acid (3-CQ), and 3-*O*-*p*-coumaroyl-caffeoylquinic acids (3-pCQ) (Fig. 1). The mobile phase consisted of a mixed solution of A solvent (0.05% phosphoric acid in water) and B solvent (water) were eluted at a flow rate of 1.00 ml/min by gradient elution program: 0—10 min, 60% A : 40% B; 10—20 min, 50% A : 50% B; 20—30 min, 40% A : 60% B; 30—35 min, 60% A : 40% B. The UV detector was fixed at 246 nm.

Assay for Peroxynitrite-Scavenging Activity Peroxynitrite ($ONOO^-$) scavenging activity was assessed by a modified Kooy's method that involved the monitoring of fluorescent rhodamine 123 produced from non-fluorescent dihydrorhodamine 123 (DHR 123) in the presence of $ONOO^-$.[14] In brief, the rhodamine buffer (pH 7.4) consisted of 50 mM sodium phosphate dibasic, 50 mM sodium phosphate monobasic, 90 mM sodium chloride, 5 mM potassium chloride, and 100 μM diethylene pentaacetic acid (DTPA). The final DHR 123 concentration was 5 μM. The buffer in this assay was prepared prior to use and placed on ice. The plant extracts were dissolved in 10% dimethyl sulfoxide (DMSO) (f.c. 5 μg/ml). The background and final fluorescent intensities were measured 5 min after treatment with and without the addition of authentic $ONOO^-$ (10 μM), dissolved in 0.3 N sodium hydroxide. The fluorescence intensity of the oxidized DHR 123 was evaluated using a microplate fluorescence reader FL 500 (Bio-Tek Instruments Inc., Winooski, VT, U.S.A.) at excitation and emission wavelengths of 480 and 530 nm, respectively. Peroxynitrite-scavenging values were calculated as the final fluorescence intensity minus the background fluorescence *via* the detection of DHR 123 oxidation and expressed as mean±S.D. L-Penicillamine was employed as a positive control.

Extraction and Fractionation of *L. stenocephala* To obtain the caffeoylquinic acid-rich extract, the MeOH extract was fractionated into the Et_2O and BuOH fractions. In brief, the dried leaves of *L. stenocephala* (2 kg) were extracted under reflux with 5 l of methanol (MeOH) for 5 h three times. The filtered total extract was evaporated under reduced pres-

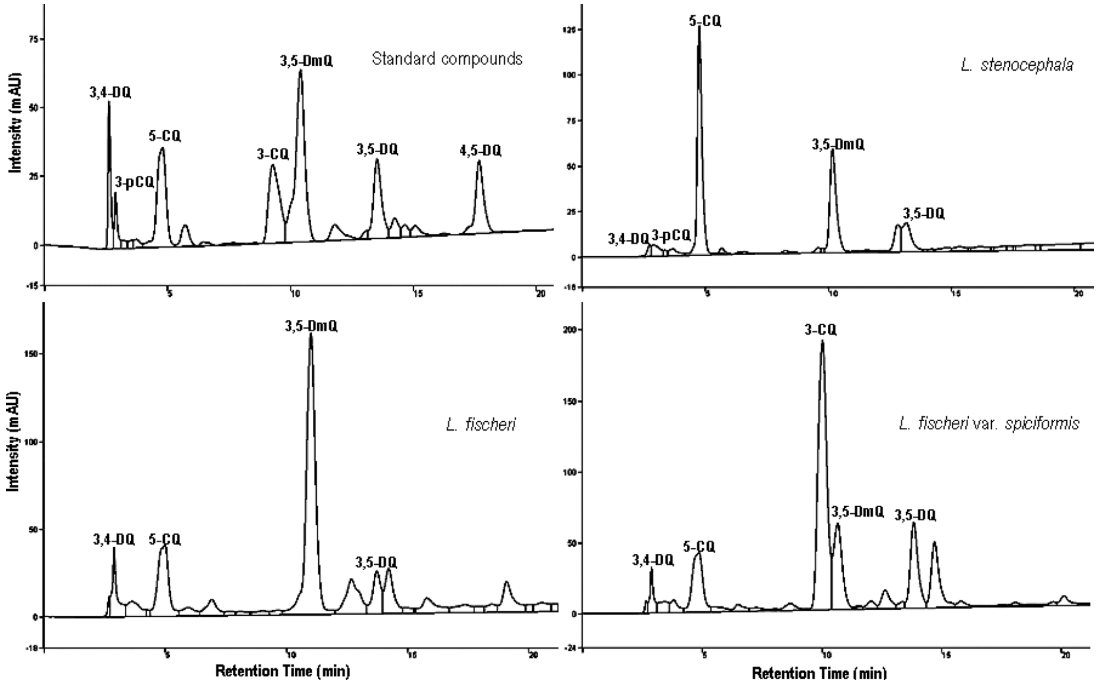

Fig. 1. HPLC Profiles of *L. stenocephala*, *L. fischeri*, and *L. fischeri* var. *spiciformis*

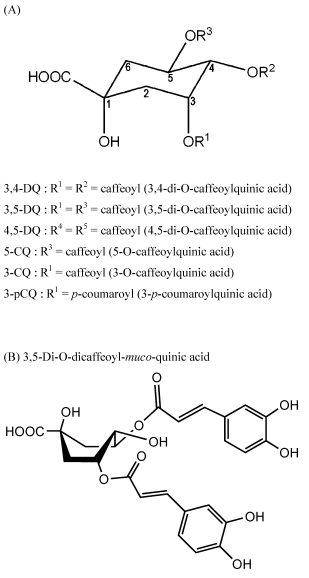

Fig. 2. Structures of Seven Standard Compounds Used for HPLC Analysis

sure on a rotatory evaporator and lyophilized to give a solid mass of MeOH extract (252 g). The MeOH extract was suspended in 2 l of H_2O and partitioned by Et_2O three times using a separating funnel. The aqueous phase was also extracted with BuOH three times. The Et_2O- and BuOH soluble portion were dried *in vacuo* to give 85 g and 93 g, respectively.

Animals Specific pathogen-free male ICR mice weighing 25±3 g were purchased from Daehan Biolink Co. (Eumseong, Republic of Korea), housed in a controlled environment at 22±3 °C and 40—60% humidity under a 12-h light/dark cycle, and cared for according to the Guide for the Care and Use of Laboratory Animals issued by the American Institute of Laboratory Animal Resources.

HCl/Ethanol-Induced Ulcer We assessed the ability of the MeOH extract of *L. stenocephala* and its BuOH and Et_2O soluble fractions together with a positive control (cimetidine) to prevent HCl/ethanol-induced ulcers in mice, as described by Mizui and Dodeuchi.[15] After oral administration (once a day for two weeks) of the test solutions dissolved in 4% tween 80, the mice were fasted for 24 h prior to the experiment. The mice were then given an oral dose of 0.2 ml of 0.3 M HCl in 60% ethanol. After 24 h the mice were sacrificed, and their stomachs were opened along the greater curvature and fixed in 2% formalin solution for 10 min. After the greater curvature was incised, the extent of gastric damage in the glandular region was evaluated according to the ulcerative lesion index.

Indomethacin/Bethanechol-Induced Ulcer This experiment was performed according to the method described by Rainsford with modifications.[16] The MeOH, BuOH, and Et_2O extracts and the omeprazole control were orally administered to mice at 100 mg/kg a day for 2 weeks. The animals were treated with indomethacin (30 mg/kg, subcutaneously (s.c.)) and bethanechol (5 mg/kg, intraperitoneally (i.p.)), fasted for 1 h, and then sacrificed. The stomachs were opened along the greater curvature and fixed in 2% formalin solution for 10 min. After the greater curvature was incised, the extent of gastric damage in the glandular region was defined as the ulcerative lesion index.

Measurement of Gastric Juice Secretion After treated mice were fasted for 24 h, they were anesthetized with ether, and then the pylorus was ligated. The volume of gastric secretion was measured using the method described by Dai and Ogle.[17] In brief, the abdomens of the anesthetized mice were opened, and the pylorus was ligated and then sealed up after the sample solutions (100 and 200 mg/kg of the MeOH extract, BuOH, and Et_2O fractions and cimetidine control) had been placed in the duodenal tract. Four hours after sealing-up of the abdomens, the mice were anesthetized with ether, the stomachs were excised, and gastric juices were collected. These were centrifuged at 3000 rpm, and then the gastric juice volumes, pH values, and acidities were measured, and total acid output was calculated. The acidity and total acid output were determined by titration versus 0.05 N NaOH using phenolphthalein as an indicator.

Statistics The statistical significance between experimental groups was analyzed using analysis of variance and Duncan's new multiple range test. $p<0.05$ was considered significant.

RESULTS

HPLC Analysis and Peroxynitrite-Scavenging Effect The leaves of three *Ligularia* species (*L. stenocephala*, *L. fischeri*, and *L. fischeri* var. *spiciformis*) were quantitatively analyzed by HPLC using seven standard caffeoylquinic acids. The three *Ligularia* species have different HPLC chromatograms (Fig. 1, Table 1). No extracts contained 4,5-DQ, *L. stenocephala* did not contain 3-CQ, *L. fischeri* did not contain 3-CQ and 3-pCQ, and *L. fischeri* var. *spiciformis* did not contain 3-pCQ. In *L. stenocephala* the three prominent peaks were 5-CQ (19.40%), 3,5-DmQ (7.78%), and 3,5-DQ (7.25%); in the *L. fischeri*, the three prominent peaks were 5-CQ (7.40%), 3,5-DmQ (11.47%), and 3,5-DQ (5.11%). *L. fischeri* var. *spiciformis* contained 3-CQ, which was not present in the other two plants. *L. stenocephala* had the highest levels of total caffeoylquinic acids at 42.20%, which was approximately two-fold of the other plants. The BuOH fraction had 94.52% caffeoylquinic acid (data not shown).

Therefore, we used this plant in animal experiments for anti-ulcerogenic effects. The IC_{50} values of the three extracts on the peroxynitrite-scavenging effects were: *L. stenocephala* (1.62±0.03 μg/ml), *L. fisceri* var. *spiciformis* (1.49±0.03 μg/ml) and *L. fischeri* (>2.0 μg/ml) (Table 2). The IC_{50} of the BuOH fraction of *L. stenocephala* was very low (IC_{50}, 0.53±0.03 μg/ml) but the Et_2O fraction was more than 2.0 μg/ml.

Effects on the MeOH Extract and Both Fractions on Gastric Ulcer Ulcerogenic indices were determined in mice with HCl/ethanol- and indomethacin/bethanechol-induced ulcers by measuring the ulcerative lesion diameter. An oral dose of HCl/ethanol caused gastric lesions with a diameter of 28.4±3.17 mm in the controls; pretreatment with the MeOH extract of *L. stenocephala* reduced this diameter at 100 and 200 mg/kg (Table 3). The BuOH fraction was more potent than the parent MeOH extract, and decreased the gastric lesion diameter to 20.4±2.12 mm and 15.3±2.06 mm at 100 and 200 mg/kg, respectively. Controls in the indomethacin/bethanechol group had a diameter of 15.1±

Table 1. Percentage of Caffeoylquinic Acids in the MeOH Extracts of *L. stenocephala*, *L. fischeri*, and *L. fischeri* var. *spiciformis*

Compound	Caffeoylquinic acids (% of extract)		
	L. stenocephala	*L. fischeri*	*L. fischeri* var. *spiciformis*
3,4-DQ	1.45±0.02[a)]	3.83±0.29	3.65±0.48
3,5-DmQ	7.78±0.14	11.47±0.32	4.09±0.16
3,5-DQ	7.25±0.18	5.11±0.24	2.89±0.16
4,5-DQ	ND[b)]	ND	ND
5-CQ	19.40±0.70	7.40±0.18	9.92±0.81
3-CQ	ND	ND	15.37±0.10
3-pCQ	6.31±0.15	ND	ND
Total % of extract	42.20±0.61	27.81±0.27	35.92±1.27
% of dried weight	7.22±0.11	4.57±0.04	7.09±0.25

a) Values represent mean±S.D. based on triplicate experiments, *b*) ND: not detected.

Table 2. IC_{50} Value of Peroxynitrite Scavenging Activity

Treatment	IC_{50} (μg/ml)
MeOH extract of *L. stenocephala*	1.62±0.03
Et_2O fraction of *L. stenocephala*	>2.0
BuOH fraction of *L. stenocephala*	0.53±0.03
MeOH extract of *L. fischeri*	>2.0
MeOH extract of *L. fischeri* var. *spiciformis*	1.49±0.03
L-Penicillamine[a)]	1.03±0.01

$ONOO^-$ scavenging activity was measured by monitoring the oxidation of DHR 123. Data are mean±S.E.M. of triplicate experiments. *a*) Used as a positive control.

Table 3. Effect of the MeOH Extract of *L. stenocephala* and Its BuOH Soluble Fraction on HCl/Ethanol- and Indomethacin/Bethanechol-Induced Gastric Ulcers in Mice

Gastric lesion model	Treatment	Dose (mg/kg, *p.o.*)	Ulcerative index (mm)
HCl–ethanol	Vehicle	—	28.4±3.17[a]
	MeOH	100	24.3±2.19[b]
		200	21.9±1.83[bc]
	BuOH	100	20.4±2.12[c]
		200	15.3±2.06[d]
	Cimetidine	100	2.59±1.43[e]
Indomethacin/bethanechol	Vehicle	—	15.1±1.46[a]
	MeOH	100	14.8±0.95[a]
		200	13.9±1.10[a]
	BuOH	100	12.4±1.09[b]
		200	10.3±0.78[c]
	Omeprazole	100	5.43±1.16[d]

Samples were administrated orally for 2 weeks before HCl/ethanol or indomethacin/bethanechol induction of gastric ulcers in mice. Data are mean±S.D. values (n=6 mice per group). Values sharing the same superscript letter are not significantly different each other ($p<0.05$) by Duncan's multiple range test.

1.46 mm; and pretreatment with MeOH decreased this diameter. The BuOH fraction was more potent than the MeOH fraction, and reduced the ulcerative index to 12.4±1.09 mm and 10.3±0.78 mm at 100 and 200 mg/kg, respectively.

Effects of the MeOH Extracts and Both Fractions on Gastric Juice Secretion The volume of gastric juice was measured using the method described by Dai and Ogle.[17] The pH values and total acid output were also measured. Oral administration of the MeOH extract reduced gastric juice volume and total acid output and increased pH in the pylorus-ligated mice at 100 and 200 mg/kg (Table 4). The BuOH fraction was more potent than the MeOH extract, suggesting that the bioactive compounds are mainly contained in the BuOH extract. However, cimetidine was more potent than the BuOH extract.

Table 4. Effect of the MeOH Extract of *L. stenocephala* and Its BuOH Soluble Fraction on the Biochemical Parameters of Gastric Juice Obtained from Pylorus-Ligated Mice

Treatment	Dose (mg/kg, *p.o.*)	pH	Gastric juice (ml/4 h)	Total gastric acid (μEq/4 h)
Vehicle	—	1.23±0.04[d]	6.74±1.21[a]	1593.7±114.6[a]
MeOH	100	1.27±0.17[cd]	5.91±1.36[ab]	1200.4±137.5[b]
	200	1.32±0.15[cd]	5.67±1.17[ab]	1016.6±114.1[c]
BuOH	100	1.65±0.13[b]	5.65±1.25[ab]	913.2±93.2[cd]
	200	1.75±0.16[b]	4.83±0.98[bc]	792.4±74.6[e]
Cimetidine	100	2.48±0.49[a]	3.71±0.97[c]	420.9±53.7[f]

Data are mean±S.D. values (n=6 mice per group). Values sharing the same superscript letter are not significantly different each other ($p<0.05$) by Duncan's multiple range test.

DISCUSSION

We analyzed the caffeoylquinic acid content of the leaves of three *Ligularia* species on HPLC chromatograms. The quantitative and quantitative analytical data were quite different among the *Ligularia* species though the reason is unclear. *L. fishceri* var. *spiciformis* and *L. stenocephala* showed similar peroxynitrite scavenging activities, but *L. fischeri* was relatively weaker. *L. stenocephala* produced high levels of caffeoylquinic acids (42.20% of the MeOH extract and 94.52% of BuOH fraction) and showed potent peroxynitrite-scavenging effect, so was selected for *in vivo* anti-ulcerogenic experiments.

Enhanced nitric oxide generation by stimulated nitric oxide synthase activity may contribute to the pathogenesis of peptic ulceration. Nitric oxide may induce tissue injury by the simultaneous generation of superoxide and nitric oxide by macrophages to yield peroxynitrite. Peroxynitrite decomposes to $\cdot OH$ and $\cdot NO_2$, which oxidizes sulfhydryl groups and reacts with metal ions.[18] Exposure of the duodenal mucosa to peroxynitrite originating in the gastric antral mucosa may induce or amplify tissue injury. Enhanced nitric oxide generation may amplify tissue damage by stimulating gastric acid secretion in several ways. Firstly, protonation of peroxynitrite is required before its decay to $\cdot OH$ and $\cdot NO_2$, which exist only in acidic pH. Secondly, nitrosation of proteins and nucleic acids is facilitated by acidic conditions, leading to impaired protein function and mutations in DNA.[19] Peroxynitrite also causes lipid peroxidation, cytotoxicity, and rapid neuronal damage,[20] and can result in atherosclerosis, diabetes mellitus, obesity, and hypocholesterolemia.[21—23] Therefore, peroxynitrite scavenging may protect against those diseases.

Peptic ulcer refers to ulcerative disorders of the upper gastrointestinal tract and involves the most proximal portion of the duodenum and the stomach, which secrete acid/pepsin, and occurs because of disturbances in the natural balance between aggressive acid/pepsin and mucosal defense/mucosal turnover.[24] Administration of HCl and ethanol produces ulcerative lesions and increases lipid peroxidation in the gastric mucosa, which plays a significant part in the pathogenesis of the mucosal lesions.[25] HCl–ethanol instillation induces se-

vere epithelial desquamation, deep mucosal necrosis and submucosal edema associated with leucocyte accumulation.[26)]

The suppression of prostaglandin synthesis by non-steroidal anti-inflammatory drugs (NSAIDS), such as indomethacin, results in increased susceptibility to mucosal injury and gastroduodenal ulceration. Indomethacin inhibits both cyclooxygenase-1 (COX-1) and COX-2 as well as the production of prostaglandins in the stomach and intestines to maintain the mucous lining of the gastrointestinal tract. Indomethacin, therefore, like other non-selective COX inhibitors, can cause peptic ulcers.[27)] Cholinomimetic agents (bethanechol) administered in association with NSAIDS have a synergistic effect on the gastric injury induced by increased secretion of acid and pepsin in the stomach.[28)]

Natural products with anti-ulcerogenic activity have been reported: flavonoids,[29)] sequiterpene,[30)] diterpenes,[31)] and saponins.[32,33)] Natural compounds with anti-*Helicobacter pylori* effects include alkaloids,[34)] sesquiterpenes,[35)] sesquiterpene lactones,[36)] flavonoids,[37)] and isoflavonoids.[38)] These reports suggest that intake of certain bioactive phytochemicals may be beneficial for gastrointestinal ulcer disease.

Dicaffeoylquinic acids are potent inhibitors of the nitration reaction of peroxynitrite against protein tyrosine residues and nitric oxide (NO) formation.[39)] The dicaffeoylquinic acids have anti-oxidative, anti-inflammatory, vascular relaxant, antimicrobial, anti-hepatotoxic and platelet anti-aggregating activities.[40)] However, this is the first report that the leaves of *L. stenocephala*, which have high levels of caffeoylquinic acids and potent peroxynitrite scavenging, have anti-ulcerogenic effects.

Acknowledgements This study was supported by Technology Development Program for Agriculture and Forestry, Ministry for Agriculture, Forestry and Fisheries, Republic of Korea.

REFERENCES

1) Aihara T., Nakamura E., Amagase K., Tomita K., Fujishita T., Furutani K., Okabe S., *Pharmacol. Ther.*, **98**, 109—127 (2003).
2) Behrman S. W., *Arch. Surg.*, **140**, 201—208 (2005).
3) Demir S., Yilmaz M., Koseoglu M., Akalin N., Aslan D., Aydin A., *Turk. J. Gastroenterol.*, **14**, 39—43 (2003).
4) Yan F. L., Wang A. X., Jia Z. J., He L., *Chinese Chem. Lett.*, **14**, 1253—1254 (2003).
5) Choi S. Z., Choi S. U., Lee K. R., *Arch. Pharm. Res.*, **27**, 164—168 (2004).
6) Yoon M. H., Cho C. W., Lee J. W., Kim Y. S., An G. H., Lim C. H., *Nat. Prod. Sci.*, **14**, 62—67 (2008).
7) Toyoda K., Yaoita Y., Kikuchi M., *Chem. Pharm. Bull.*, **12**, 1555—1558 (2005).
8) Murae T., Tanahashi Y., Takahashi T., *Tetrahedron*, **24**, 2177—2181 (1968).
9) Yan F. L., Wang A. X., Jia Z. J., He L., *Chinese Chem. Lett.*, **15**, 423—424 (2004).
10) Kwon H. C., Jung C. M., Shin C. G., Lee J. K., Choi S. U., Kim S. Y., Lee K. R., *Chem. Pharm. Bull.*, **48**, 1796—1798 (2000).
11) Choi J. W., Park K., Lee K. T., Park K. K., Kim W. B., Lee J. H., Jung H. J., Park H. J., *Nat. Prod. Sci.*, **10**, 182—189 (2004).
12) Kim K. H., Lee K. H., Choi S. U., Kim Y. H., Lee K. R., *Arch. Pharm. Res.*, **31**, 983—988 (2008).
13) Kim K. H., Kim Y. H., Lee K. R., *Bioorg. Med. Chem. Lett.*, **17**, 6739—6743 (2007).
14) Kooy N. W., Royall J. A., Ischiropoulos H., Beckman J. S., *Free Radic. Biol. Med.*, **16**, 149—156 (1994).
15) Mizui T., Dodeuchi M., *Jpn. J. Pharmacol.*, **33**, 939—945 (1983).
16) Rainsford K. D., *Agents Actions*, **5**, 553—558 (1975).
17) Dai S., Ogle C. W., *Life Sci.*, **12**, 505—512 (1973).
18) Radi R., Beckman J. S., Bush K. M., Freeman B. A., *J. Biol. Chem.*, **266**, 4244—4250 (1991).
19) Rachmilewitz D., Karmeli F., Eliakim R., Stalnikowicz R., Ackerman Z., Amir G., Stamler J. S., *Gut*, **35**, 1394—1397 (1994).
20) Haenen G. R., Paquay J. B., Korthouwer R. E., Bast A., *Biochem. Biophys. Res. Commun.*, **236**, 591—593 (1997).
21) Korda M., Kubant R., Patton S., Malinski T., *Am. J. Physiol. Heart Circ. Physiol.*, **295**, 1514—1521 (2008).
22) Patcher P., Obrosova I. G., Mabley J. G., Szabo C., *Curr. Med. Chem.*, **12**, 267—275 (2005).
23) Drel V. R., Patcher P., Vareniuk I., Pavlov I., Lyzogulbov V. V., Grovez J. T., Obrosova I. G., *Eur. J. Pharmacol.*, **569**, 48—58 (2007).
24) McGuigan J. E., "Harrison's Principles of Internal Medicine," 13th ed., Vol. 2, ed. by Kurt J. I., Eugene B., Margin J. B., Wilson J. D., Karper D., 1994, p. 1363.
25) Anandan R., Rekha R. D., Saravanan N., Devaki T., *Fitoterapia*, **70**, 498—501 (1999).
26) Kurebayashi Y., Ikeda T., Osada Y., *Jpn. J. Pharmacol.*, **46**, 17—25 (1988).
27) Asay S., Tarnawski A. S., Dubois A., *Prostag. Other Lipid Mediat.*, **61**, 105—124 (2005).
28) Rainsford K. D., *Biochem. Pharmacol.*, **27**, 1281—1289 (1978).
29) Lewis D. A., Fields W. N., Shaw G. P., *J. Ethnopharmacol.*, **65**, 283—288 (1999).
30) Yesilada E., Gurbuz I., Bedir E., Tatli I., Khan I. A., *J. Ethnopharmacol.*, **95**, 213—219 (2004).
31) Melo P. S., Duran N. D., Hiruma-Lima C. A., Souza-Brito A. R. M., Haun M., *J. Ethnopharmacol.*, **87**, 169—174 (2003).
32) Yesilada E., Takaishi Y., *Phytochemistry*, **51**, 903—908 (1999).
33) Yoshikawa M., Murakami T., Kishi A., Kageura T., Matsuda H., *Chem. Pharm. Bull.*, **49**, 863—870 (2001).
34) Carroll A. R., Ngo A. N., Quinn R. J., Redburn J., Hooper N. A., *J. Nat. Prod.*, **68**, 804—806 (2005).
35) Ochi T., Shibata H., Higuti T., Kodama K., Kusumi T., Takaishi Y., *J. Nat. Prod.*, **68**, 819—824 (2005).
36) Konstatinopoulou M., Karioti A., Skaltsas S., Skaltsa H., *J. Nat. Prod.*, **66**, 699—702 (2003).
37) Fukai T., Marumo A., Kanda T., Terada S., Nomura T., *Life Sci.*, **71**, 1449—1463 (2002).
38) Takashima J., Chiba N., Yoneda K., Ohsaki A., *J. Nat. Prod.*, **65**, 611—613 (2002).
39) Olmos A., Guner R. M., Recio M. C., Rios J. L., Gil-Benso R., Marnez S., *Arch. Biochem. Biophys.*, **475**, 66—71 (2008).
40) Zhao Y., Zhao J., *Zhonguo Zhongyao Zazhi*, **31**, 869—874 (2006).