Biomolecules & Therapeutics, **18(4)**, 433-441 (2010)
www.biomolther.org

ISSN: 1976-9148(print)/2005-4483(online)
DOI: 10.4062/biomolther.2010.18.4.433

Anti-Gastritis and Anti-Oxidant Effects of *Chenopodium album Linne* Fractions and Betaine

Pitna KIM, and Choon Sik JEONG*

College of Pharmacy, Duksung Women's University, Seoul 132-714, Korea

(Received July 1, 2010; Revised July 1, 2010; Accepted August 6, 2010)

Abstract − *Chenopodium album Linne* (CAL) is a fast-growing weedy annual plant. The leaves and young shoots may be eaten as a leafy vegetable. In oriental medicine, CAL has been used for treatment of skin disease, fever, stomach ache, toothache, and paralysis. After a preliminary screening of CAL ethanol extract and its fractions obtained from CAL leaves for anti-gastritic and anti-*Helicobacter pylori* (*H. pylori*) activity, the butanol (BuOH) fraction was found to have the most significant effect. We also examined antioxidative properties of the total CAL extract and its fractions, and also betaine as an ingredient of the BuOH fraction. To investigate the antioxidant effects of CAL on gastritis, the reducing power, free radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH), and lipid peroxidation effects were determined. Additionally, the BuOH fraction reduced cell viability in a concentration dependent manner in human gastric cancer cell lines. The results of this study revealed that CAL has excellent antioxidant activity, and may be useful in treating gastritis and gastric cancer.

Keywords: *Chenopodium album Linne*, Betaine, Anti-gastritis, Anti-oxidant, Anti-*Helicobacter pylori*, Cell viability

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a cause of chronic gastritis (NIH Consensus Conference, 1994) and gastric ulcers (Blaser, 1992), and is a risk factor for stomach cancer (Parsonnet *et al.*, 1991). *H. pylori* is spread throughout the entire world; 64-95% of chronic gastritis patients and 35-86% of gastric ulcer patients are infected with *H. pylori* (Megraud and Lamouliatte , 1992). From the perspective of the "Balance Theory," according to which gastric mucous membrane damage in gastric ulcers is caused by an imbalance between aggressive and defensive factors in the stomach, *H. pylori* is believed to alter the defensive factors and lead to excessive acid production (Shay *et al.*, 1945; Leunk *et al.*, 1988). The cause of gastric mucous membrane damage related to *H. pylori* was initially explained by toxic factors such as cytotoxin, urease, and ammonia. However, it has been recently reported that phagocytes, which produce excessive amounts of reactive oxygen metabolites, are also partially responsible. Unlike *H. pylori*, cimetidine, ranitidine, and proton pumps (H^+/K^+ ATPases) are among the types of H_2-antagonists which are the most well-known inhibitors of aggressive factors and stimulators of defensive factors (Robert *et al.*, 1979; Chandranath *et al.*, 2002). Research on platelet activating factor antagonists, phosphodiesterase inhibitors, selective M_1-antagonists, gastrin inhibitors, and cytoprotective drugs is onging (Uchida *et al.*, 1989), and it is predicted that future gastritis and gastric ulcer research will be focused on *H. pylori* and antioxidant and anti-inflammatory substances. Thus, drug development research, along with the existing antacid medications, should shift focus to the pathogenesis of gastric ulcers caused by *H. pylori* and the development of anti-inflammatory drugs.

Chenopodium album Linne (CAL) was selected for this study, since it has long been used as a folk remedy due to its effectiveness in treating various illnesses such as neuralgia, gastralgia, and hepatocirrhosis. Young leaves of CAL, an annual plant of Chenopodiaceae, are edible, and contain essential oils, amino acids, ascorbic acid, saponin, mucus, ferulic acid, vanillic acid, betaine, lyciun, barparum, and propylamin. In oriental medicine, CAL is called "Yeoh" and is gathered from May to June., Both dried and fresh

*Corresponding author
Tel: +82-2-901-8382 Fax: +82-2-901-8386
E-mail: choonsik@duksung.ac.kr

decoct of "Yeoh" have been used for treatment of dysentery, strengthening of the stomach, alleviation of fever, detoxification, prevention of stroke, alleviation of toothache, stomach ache, labor pains, treatment of tuberculosis, and tonics. Juice of CAL leaves can be applied on the skin to treat and alleviate symptoms of Prurigo nodularis, scabies, eczema, hemorrhoids, and poisonous insect bites. Betaine is contained in the CAL BuOH fraction and was named after its discovery in sugar beets (*beta vulgaris*) during the 19th century. This substance is widely distributed in micro-organisms, plants, and animals. Betaine is a white crystal with a formula of $C_5H_{11}NO_2$ (Fig. 1) and has a slight odor and sweetness. Betaine is anti-glycemic, lipotropic, and active in detoxification and cellular replication. Hydrochloric acid, released from betaine-HCl, may help alleviate symptoms of lack of gastric acid. Potential uses of CAL were investigated for the treatment of acute and chronic gastritis. The HCl/ethanol-induced mucosal membrane lesion model was used for acute gastritis, and the indomethacin induced gastric lesion model was used for chronic gastritis. The antibacterial activity on *H. pylori* and the cytotoxicity on SNU638 and AGS cells, along with the antioxidant activities of CAL EtOH extract, its extract fractions, and betaine were also investigated. Therefore, by confirming its antioxidative mechanism, the study sought to provide scientific rationale for using a natural substance such as CAL.

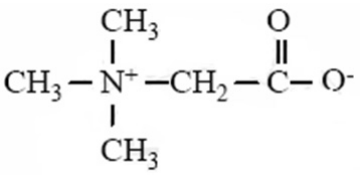

Fig. 1. Chemical structure of betaine.

MATERIALS AND METHODS

Reagents

Dimethyl sulfoxide (DMSO), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 3-(4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide (MTT), trypan blue, cimetidine, hydrotalcite and ampicillin were obtained from Sigma (Sigma-Aldrich Inc., MO, USA). Cell culture media and reagents, including Hank's balanced salt solution, RPMI 1640, fetal bovine serum (FBS), penicillin/streptomycin, and trypsin-EDTA were obtained from GIBCO (Invitrogen Inc., NY, USA). HCl, EtOH, and other solvents were purchased from Duksan pure Chemical Co. Ltd. (Kyunggi-do, Korea). All other reagents were of pharmaceutical or analytical grade.

Laboratory equipment

Equipment included: evaporator (Eyela), pH meter (IQ Scientific Instruments, Inc), Clean Bench (Johnsam Co.), CO_2 incubator (Forma Scientific), water bath (Vision), inverted microscope (Olympus), autoclave (Duksan Chem. Co.), micropipette (Gilson Co.), centrifuge 5810R (Eppendorf), high speed centrifuge (Sorvall RT-6000), AnaeroPack Campylo (Mitsubishi Gas Chemical Co., Inc.), liquid nitro-

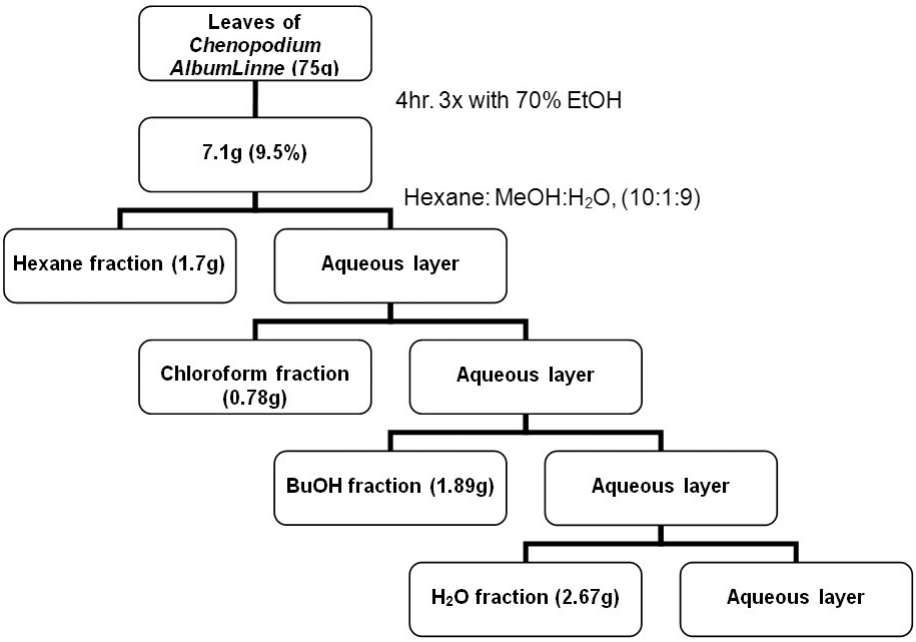

Fig. 2. Extraction and fractionation from CAL.

gen Dewars (CHART MVE), UV-spectrophotometer (Agilent Technologies Inc., CA, USA), UV-spectrophotometric plate reader (ASYS UVM340), etc.

Preparation of extract and fractions

The method for extraction and subsequent fractionation of CAL (provided from National Products Research Institute, Seoul National University, Korea) is shown in Fig. 2. Leaves of CAL were extracted with 70% EtOH in a reflux condenser. The CAL 70% EtOH extract was concentrated under reduced pressure to give a residue (74 g) which was further fractionated using Hexane:MEOH:H_2O (10:1:9). Final extract yields, obtained by using solvents of increasing polarity were: hexane (0.78 g), chloroform (0.78 g), n-buthanol (BuOH) (1.89 g) and H2O (2.67 g).

Analysis of active ingredient

The analysis of active ingredient was conducted using liquid chromatography with a UA-5® Detector and a TSK-GEL-NH_2 column. The method used a mobile phase consisting of acetonitrile:water (85:15) at flow rate of 0.8 ml/min, and a UV detector wavelength setting of 210 nm.

Animals

Male Sprague-Dawley rats, weighing 190-210 g, were purchased from Samtako, Kyunggi-do, Korea, and were acclimatized to standard laboratory conditions (22 ± 2°C, 55 ± 5% humidity and 12 h light/dark cycle) for 14 days in the animal facility in Duksung Women's University. The experimental procedures for rats were conducted in accordance with the Guidelines of the Care and Use of Laboratory Animals, Duksung Women's University. The animals were allowed free access to food (standard pellet diet) and water ad libitum. The entire study was conducted in compliance with the Testing Guidelines for Safety Evaluation of Drugs (Notification No. 1999-61) issued by the Korea Food and Drug Administration, the Good Laboratory Practice Regulations for Non-clinical Laboratory Studies (Notification No. 2000-63) issued by the Korea Food and Drug Administration, and the Principles of Good Laboratory Practice issued by the Organization for Economic Cooperation and Development.

In vivo experiments

HCl/EtOH-induced mucosal membrane lesions: After 24 hours of fasting, with free access to water prior to the experiment, samples were administered orally to the rats. Thirty minutes later, 1 ml of HCl/EtOH solution (150 mM HCl in 60% EtOH) was administered orally. After 1 hour fasting, each animal was sacrificed by ether inhalation and the stomach was excised, inflated by injecting 2 ml of normal saline and then fixed for 30 min in 2% formalin solution. The stomach was incised along the greater curvature, and the glandular portion was examined for hemorrhage. The length (mm) of each lesion was measured under the dissecting microscope (10X), and a total value was expressed as a lesion index. (Mizui and Dodeuchi, 1983). Cimetidine and hydrotalcite were used as positive control drugs.

Indomethacin induced gastric lesion: Using the method of Kasuya *et al.* (1979), rats were fasted for 24 hours with free access to water before the experiment. The sample was orally dosed and 30 min later, indomethacin (35 mg/kg suspended in 0.5% CMC) was injected subcutaneously. The animals were sacrificed 7 hr after the indomethacin injection, and the excised stomach was fixed for 30 min in 2% formalin solution. The stomach was incised along the greater curvature and examined for the presence of hemorrhage in the glandular portion. The length (mm) of each lesion was measured and a total value was expressed as a lesion index. Cimetidine was used as a positive control drug.

In vitro experiments

Anti-*H. pylori* activity: *H. pylori* strain (ATCC 43504) was obtained from American Tissue Culture Collection (ATCC, Rockville, MD, USA). The inhibitory effect of samples on the growth of *H. pylori* was investigated using a modification of the method described by Bae *et al.* (2001) and Kim *et al.* (2003). A six hundred microliter of sample was mixed with 5.4 ml of brucella agar medium containing 7% horse serum in a petri dish. *H. pylori* (5×10^5 CFU) was seeded in this media and then incubated for 3 days at 37°C incubator - (AnaeroPak Campylo: 85% N_2, 10% CO_2, 5% O_2). Viability of *H. pylori* was determined by colony counts after 3 days incubation. Ampicillin was used as a positive control.

Cell culture and cytotoxicity assay: SNU638 gastric cancer cells and AGS gastric cancer cells were obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea). These cells were cultured with RPMI-1640 containing 10% FBS, penicillin (100 units/ml), and streptomycin (100 lg/ml) in a 5% CO_2 humidified incubator at 37°C. For subculture, SNU638 and AGS cells were rinsed twice with phosphate buffered saline (PBS, pH 7.4) to remove all traces of serum (which can inhibit trypsin) and were subdivided using 0.05% trypsin with 0.53 mM EDTA.

Cytotoxicity to SNU638 and AGS cells, (gastric cancer cell lines), was examined using the MTT assay. Cells were seeded at 1×10^4 cells/well in 96-well culture plates (Corning

Inc., USA), and were cultured for 24 h at 37°C in a 5% CO_2 humidified incubator. The samples were added to the plate and incubated for 24 h. MTT was added at a final concentration of 0.5 mg/ml and samples were incubated for 4 h at 37°C. After discarding all media from the plates, 100 ul of dimethyl sulfoxide (DMSO) was added to all wells. The plates were held for 5 min at room temperature with shaking to achieve complete dissolution of formazan. The absorbance of the MTT formazan was determined at 540 nm using a UV-spectrophotometric plate reader (Choi *et al.*, 2004).

Antioxidant activities

Free radical scavenging activity: One milliliter of DPPH (150 μM) in MEOH was added to 4 ml of extracts, and the mixture was stirred. After 30 min incubation at room temperature, the absorbance of the mixture was read against a blank at 520 nm and the scavenging DPPH free radical was monitored. The concentration providing 50% inhibition (IC_{50}) was determined from the graph plotting the percentage inhibition against concentration. L- Ascorbic acid was used as a positive control (Lee *et al.*, 2005).

Reducing power: The reducing power was determined according to the method of Oyaizu (1986). Samples were mixed with 500 μl of 0.05 M phosphate buffer (pH 6.6) and 500 ul of 1% $K_3Fe(CN)_6$ (Sigma T-3667) and the mixture was incubated at 50°C for 20 min. 500 ul of 10% TCA (Trichloroacetic acid, Sigma T-6399) was the added and the mixture was centrifuged at 3,000 rpm for 10 min. The 500 ml supernatant layer was added to 500 ml of distilled water and 100 ul of 0.1% $FeCl_3$ (Sigma F1513). The absorbance of the mixture was determined at 700 nm using UV-spectrophotometry.

Lipid peroxidation: The ability of the extract to inhibit the bleaching of the β-carotene-linoleic acid emulsion was determined using a modification of the method described by Koleva *et al.* (2002). 0.3 mg β-carotene dissolved in 3 ml chloroform, 40 mg of linoleic acid and 400 mg of Tween40 were transferred into a round-bottom flask. Following removal of the chloroform under nitrogen, 100 ml distilled H_2O was added and the resulting mixture was vigorously stirred for 30 min, after which, 5 ml aliquots of the emulsion were transferred to tubes containing either 200 ul of dissolved extract or 200 ul of positive control. Each sample was placed in a water bath at 40°C for a period of 1hr and the absorbance at 470 nm was recorded. The data is presented as antioxidant activity (%) values, calculated using $[1-(Ac-As)/Ac]\times100$.

Statistical analysis: All experiments were performed in triplicate. Data was analyzed using the Student's *t*-test. *p*-values <0.05 were considered statistically significant. When gastric lesions were induced by various methods, inhibitory effects on gastritis and gastric ulcer were determined as an inhibition ratio (%).

$$\text{Inhibition ratio (\%)} = \frac{\text{lesion length (control)} - \text{lesion length (drug)}}{\text{lesion length (control)}} \times 100$$

RESULTS

Effects of CAL EtOH extract and its fractions on HCl/EtOH-induced gastric lesions

The effect of CAL extract on the HCl/EtOH induced lesion was investigated (Table I). The lesion index of the control group was 93.2 ± 14.39 mm. The CAL extract (500 mg/ml) decreased the lesion index by approximately 71.0%, which was better than or equivalent to results using cimetidine (150 mg/kg) and hydrotalcite (150 mg/kg) as positive controls. The effects of CAL fractions on the HCl/EtOH induced lesion were investigated (Table II). The lesion index of the control group was 90.9 ± 9.31 mm. Treatment with individual CAL fractions including: hexane fraction (150 mg/kg), chloroform fraction (30 mg/ml), BuOH fraction (150 mg/kg), and H_2O fraction (200 mg/ml)

Table I. The effects of CAL EtOH extract on HCl · EtOH induced gastric lesion in rats

Material	Dose (mg/kg)	Lesion index (mm)	Inhibition rate (%)
Control	–	93.2 ± 14.39	–
	300	35.0 ± 12.38[b]	62.4
EtOH extract	500	27.0 ± 19.97[b]	71.0
Cimetidine	150	56.5 ± 23.33[a]	39.4
Hydrotalcite	150	29.5 ± 5.07[b]	68.4

The values are means ± S.E.
[a]$p<0.01$, [b]$p<0.001$ compared to the control group (n=6).

Table II. The effects of CAL fractions on HCl · EtOH induced gastric lesion in rats

Material	Dose (mg/kg)	Lesion index (mm)	Inhibition rate (%)
Control	–	90.9 ± 9.31	–
Hexane fraction	150	57.0 ± 10.23[a]	37.3
Chloroform fraction	30	58.0 ± 5.29[a]	36.2
BuOH fraction	150	47.8 ± 5.26[b]	47.4
H_2O fraction	200	48.4 ± 5.07[b]	46.7
Cimetidine	150	53.5 ± 8.23[a]	41.1
Hydrotalcite	150	26.2 ± 6.46[b]	71.2

The values are means ± S.E.
[a]$p<0.01$, [b]$p<0.001$ compared to the control group (n=6).

Table III. The effects of CAL EtOH extract on indomethacin induced gastric lesion in rats

Material	Dose (mg/kg)	Lesion index (mm)	Inhibition rate (%)
Control	—	14.5 ± 2.95	—
	300	9.4 ± 3.45[a]	35.0
EtOH extract	500	7.0 ± 3.00[b]	51.7
Cimetidine	150	6.0 ± 3.00[b]	58.6

The values are means ± S.E.
[a]$p < 0.05$, [b]$p < 0.001$ compared to the control group (n=6).

Table IV. The effects of CAL fractions on indomethacin induced gastric lesion in rats

Material	Dose (mg/kg)	Lesion index (mm)	Inhibition rate (%)
Control	—	26.4 ± 6.09	-
Hexane fraction	150	17.7 ± 3.67[a]	33.0
Chloroform fraction	30	16.0 ± 8.25[a]	39.3
BuOH fraction	150	11.0 ± 6.20[b]	58.3
H_2O fraction	200	11.4 ± 9.07[b]	56.8
Cimetidin	150	7.00 ± 3.37[b]	73.5

The values are means ± S.E.
[a]$p < 0.01$, [b]$p < 0.001$ compared to the control group (n=6).

significantly decreased the lesion index by approximately 37.3%, 36.2%, 47.4% and 46.7%, respectively.

Effects of EtOH extract and its fractions on indomethacin induced gastric lesions

The effect on indomethacin induced gastric lesion is shown in Table III. The lesion index of the control group was 14.5 ± 2.95 mm. The CAL EtOH extract showed a significant inhibition of -51.7% at a dose of 500 mg/kg p.o. Cimetidine, at 150 mg/kg, inhibited by -58.6%. As shown in Table IV, all CAL fractions produced a significant inhibition. The congestion is observed in the gastric epithelium of the control group. The lesion index of the control group was 26.4 ± 6.09 mm, while the hexane fraction (150 mg/kg), chloroform fraction (30 mg/kg), BuOH fraction (150 mg/kg) and H2O fraction (200 mg/kg) showed decreased lesion indexes of -33.0%, 39.3%, 58.3% and 56.8%, respectively.

Analysis of betaine by HPLC-UV

Approximately 10 mg of betaine standard was accurately weighed. and standardized by dissolution in 1 ml DMSO. An analytical determination of betaine was done using TSK-GEL-NH_2 column. The method used a mobile phase consisting of acetonitrile:water (85:15), a flow rate of 0.8 ml/min, and a detector wavelength of 210 nm. Using the above conditions, peaks of sample (10 μg) and betaine standard (10 μg) were detected using liquid chromatography (Fig. 3).

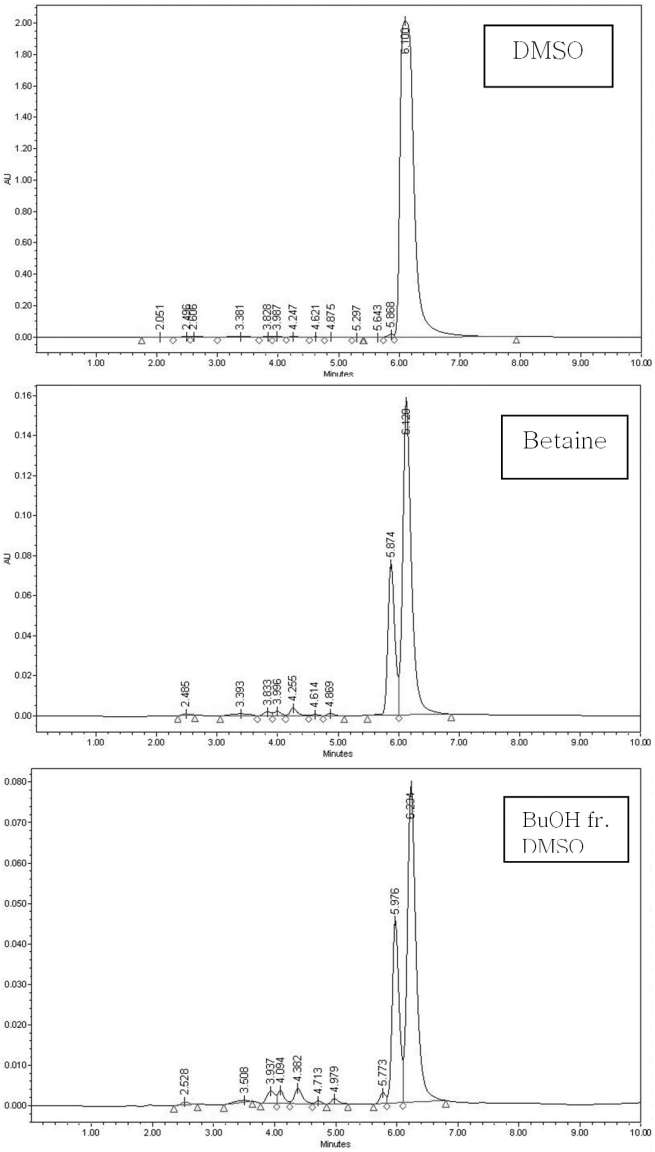

Fig. 3. HPLC of CAL BuOH fraction and betaine.

Anti-*H. pylori* effects of CAL EtOH extract and its fractions

The CAL EtOH extract and its fractions were used to run in vitro experiments on *H. pylori* survival. We found that CAL EtOH extract completely inhibited the colonization of *H. pylori* at a dose of 100 μg/ml, and this effect was equivalent to that of ampicilin (10 μg/ml) (Table V). The hexane fraction, chloroform fraction, BuOH fraction and H_2O fraction also completely inhibited the colonization of *H. pylori* at the same dose of 250 μg/ml.

Table V. Colonization inhibiting effect of CAL ethanol extract and its fractions on *H. pylori*

Extract	Dose (ug/ml)	Colonization
Control	−	++++
EtOH extract	10	+++
	50	+
	100	−
Hexane fraction	10	++++
	50	+++
	100	++
	250	−
Chloroform fraction	10	++++
	50	+++
	100	++
	250	−
BuOH fraction	10	++++
	50	++++
	100	+++
	250	−
H_2O fraction	10	++++
	50	+++
	100	++
	250	−
Ampicillin	1	++
	10	−

−: none, +: colonies (0-2×10^5 CFU), ++: colonies (2-4×10^5 CFU), +++: colonies (4-5×10^5 CFU), ++++: >5×10^5 CFU.

Cytotoxicities of CAL EtOH extract and its fractions

Cytotoxicity of the CAL EtOH extract and its fractions was assessed by measuring the cell viability of SNU638 and AGS human gastric cancer cell lines (Fig. 4, 5). The hexane fraction, chloroform fraction, BuOH fraction and H_2O fraction demonstrated cell viability in SNU638 gastric cancer cells 88.04%, 88.26%, 84.53% and 82.26%, respectively, and showed cell viability 78.80%, 34.25%, 71.49% and 76.32% respectively, in AGS gastric cancer cells at a dose of 100 μg/ml. CAL extract and its fractions demonstrated cytotoxicity in a concentration dependent manner.

Antioxidant activities of CAL EtOH extract and its fractions

The scavenging effect of CAL EtOH extract and its fractions on DPPH free radical was investigated by using 0.15 ×10^{-4} M/ml DPPH. As shown in Table VI, the IC_{50} value of L-ascorbic acid, used as a control, was <5 μg/ml. The BuOH fraction showed a free radical scavenging activity (IC_{50}=86.8 μg/ml), and was superior to other fractions. However, the EtOH extract, hexane fraction, chloroform fraction and H_2O fraction had low radical scavenging activ-

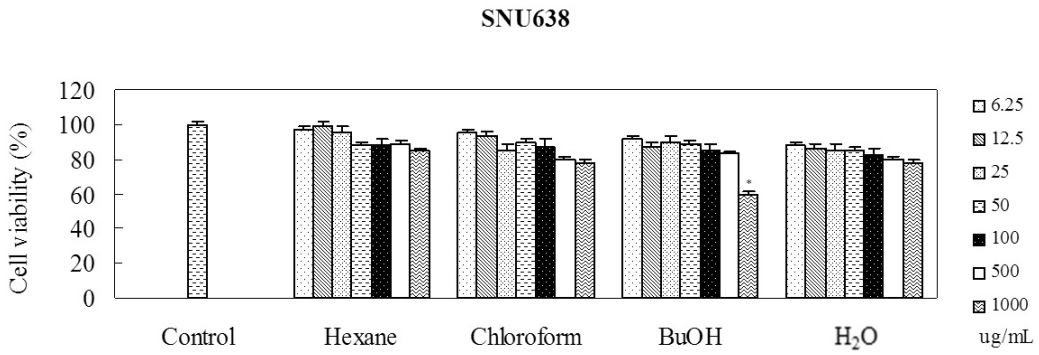

Fig. 4. MTT assay of CAL fractions against SNU 638 cells. The values are means ± S.E. *$p<0.05$ compared to the control group.

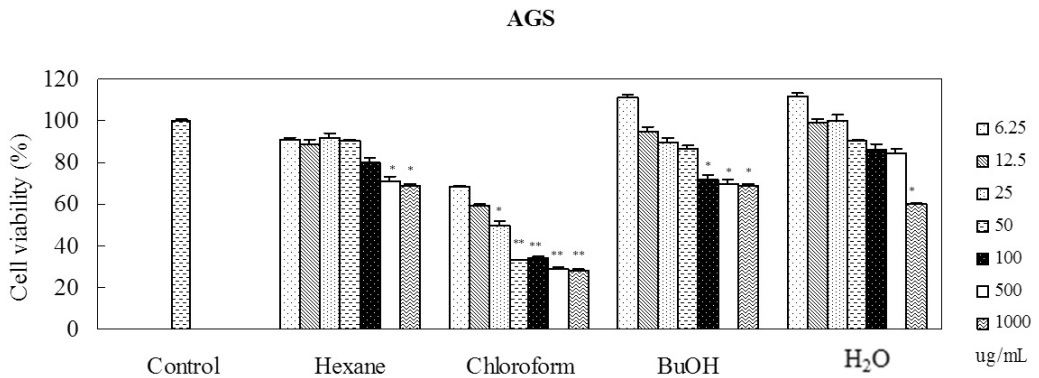

Fig. 5. MTT assay of CAL fractions against AGS cells. The values are means ± S.E. *$p<0.05$, **$p<0.01$ compared to the control group.

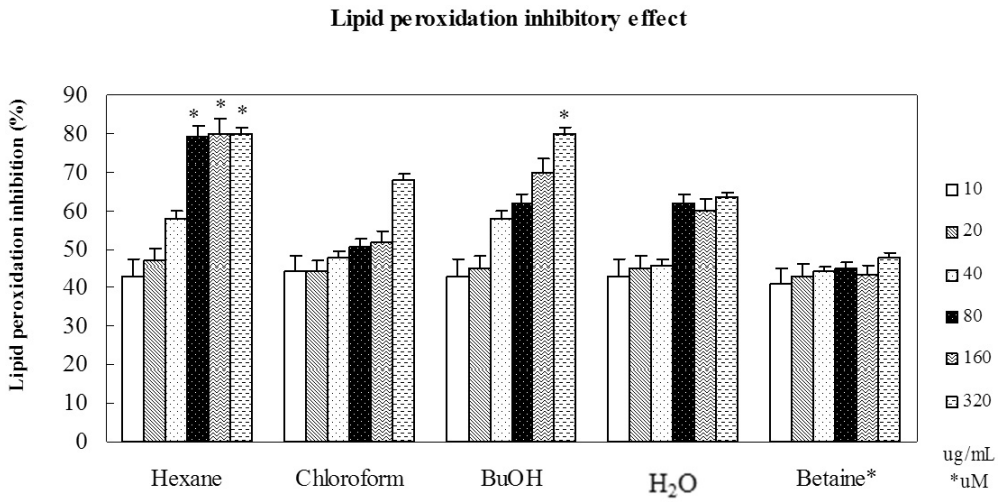

Fig. 6. Lipid peroxidation inhibitory activity of CAL fractions. The values are means ± S.E. *$p < 0.05$ compared to the control group.

Table VI. Free radical scavenging activities of CAL ethanol extract and its fractions

Material	IC_{50} (μg/ml)
Control	–
EtOH extract	>160
Hexane fraction	>160
Chloroform fraction	>160
BuOH fraction	86.8
H_2O fraction	>160
L-Ascorbic acid	<5

ities, with an IC_{50} >160 μg/ml. Reducing power, determined by Fe^{3+} reduction, is shown in Table VII. Pyrogallol (100 μg/ml) had a high reducing power, 1.682 ± 0.003 as a control. The EtOH extract had a lower reducing capacity than pyrogallol (100 μg/ml), but it had a higher reducing power than the BuOH fraction and betaine. The inhibition of lipid peroxdation by CAL fractions was investigated (Fig. 6). Both the hexane fraction and the BuOH fraction (320 μg/ml) showed high lipid peroxidative inhibitory effects of -80%. Betaine showed an inhibitory effect of -50%, regardless of concentration.

Table VII. Reducing power of CAL EtOH extract, BuOH fraction, and betaine

Material	Con (mg/ml)	(Abs 700 nm)
Control		0.096 ± 0.005
Pyrogallol 100		1.682 ± 0.003
Ascorbic acid 100		0.645 ± 0.010
EtOH extract	6.25	0.213 ± 0.013
	12.5	0.195 ± 0.108
	25	0.231 ± 0.097
	50	0.293 ± 0.002
	100	0.322 ± 0.039
	500	0.339 ± 0.016
	1,000	0.371 ± 0.011
BuOH fraction	10	0.071 ± 0.037
	20	0.067 ± 0.040
	40	0.079 ± 0.046
	80	0.086 ± 0.056
	160	0.095 ± 0.066
	320	0.157 ± 0.083
Betaine[a]	10	0.054 ± 0.012
	20	0.054 ± 0.010
	40	0.062 ± 0.012
	80	0.071 ± 0.011
	160	0.081 ± 0.010
	320	0.120 ± 0.026

[a]μM

DISCUSSION

A HCl/EtOH-induced gastric lesion model was used to study the stomach protective effects of CAL, a plant that has traditionally been associated with gastric and digestive functions. The principle of the HCl/EtOH-induced gastric lesion is based on the administration of free HCl as an aggressive factor. Using this model, it is possible to predict secretion of mucus in response to gastric acid, an aggressive factor, and the existence of defensive factors. Using HCl-EtOH induced gastric lesions, it was revealed that cimetidine, a positive control substance, inhibited gastric damage by approximately 39.4%, compared with the control, at a dose of 150 mg/kg. CAL EtOH extract inhibited gastric damage by 71.0% at a dose of 500 mg/kg and showed a significantly higher effect than cimetidine. In addition, CAL fractions showed significant inhibitory effects of 35-45%. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as indomethacin or aspirin, cause gastritis when taken at a large dosage because they hinder the

synthesis of prostaglandins. It has been reported that gastric damage occurs because of the mucus damage from excessive gastric motility caused by indomethacin, and the hindered synthesis of prostaglandin further exacerbates the gastric damage. The study therefore focused on studying the mechanism of anti-gastritis drugs by using an experimental model reflecting gastritis caused by these NSAIDs. When compared with the control group, CAL EtOH extract significantly inhibited indomethacin-induced gastric damage by 51.7% after a subcutaneous injection of 500 mg/kg. The CAL BuOH fraction also showed a significant inhibitory effect on gastric damage of 58.3%.

After observing that CAL EtOH extract and its fractions are effective in animal experiments, an analysis of betaine was performed, since it is an ingredient of the most significantly effective BuOH fraction. The CAL BuOH fraction was isolated and its active ingredient, betaine, was identified by HPLC-UV; CAL's antibacterial activity against *H. pylori* was then examined. *H. pylori* is the major cause of chronic gastritis and gastric ulcers. Gastric mucous of a patient infected with *H. pylori* shows acute and chronic inflammation, brought on by gastric mucus cell damage and necrosis, as well as degeneration of epidermal cells. Damage to epidermal cells is known to be caused by the bacteria's secretion or an inflammatory reaction to *H. pylori*. In the antibacterial assays against *H. pylori*, CAL EtOH extract completely inhibited the formation of bacterial colonies at a dose of 100 μg/ml. Also, all fractions showed a complete inhibition of colony formation at a dose of 250 μg/ml. CAL EtOH extract appears to strongly inhibit proliferation of *H. pylori*, thus alleviating gastric cancer induction mechanisms such as degradation of carcinogen resistance, induction of mucosal hyperproliferation, increase of carcinogenic N-nitroso compound, and decreased ascorbic acid secretion, while effectively preventing secondary infections.

In addition, effects on gastritis and gastric ulcers were confirmed by observation of radical scavenging activity, the DPPH test, lipid peroxidation, the reducing power test, and lipid peroxidation using the β-catrotene-linoleic acid system. Free radicals play an important role in cell and tissue damage in many organisms, and there have been reports that point to free radicals as the cause of gastric mucus damage due to EtOH, stress, aspirin, ischemia/reperfusion injuries, NSAIDs, as well as *H. pylori* (Leirisalo-Repo *et al.*, 1993). Lipid peroxidation was revealed to be a significant factor in gastric mucus damage (Yoshida *et al.*, 1996), and causes cell membrane destruction and damage by influencing unsaturated fatty acids, and thus hindering normal cell membrane functions. In a test for inhibition of lipid peroxidation, the BuOH fraction exhibited the strongest inhibitory activity against β-catrotene/linoleic acid, with an 80% inhibition at a dose of 320 μg/ml.

This study confirmed that the anti-gastritis effect of CAL is due to its anti-inflammatory effects against *H. pylori* and an increase of antioxidant activitiy. CAL also exhibited cytotoxicity against gastric cancer cells. Thus, we were able to confirm the prospect of using CAL in the development of medications for treatment of gastritis and gastric cancer. Continued research on using medicinal plants for developing functional foods, as a preventive measure against various diseases, will surely contribute to human welfare.

ACKNOWLEDGMENTS

This research was supported by Duksung Women's University Research Grants 2009.

REFERENCES

Bae, E. A., Han, M. J., Baek, N. I. and Kim, D. H. (2001). *In vitro* anti *Helicobacter pyloriactivity* of panaxtriol isolated from ginseng. *Arch. Pharm. Res.* **24**, 297-299.

Blaser, M. J. (1992). Hypotheses on the pathogenesis and natural history of Helicobacter pylori - induced inflammation. *Gastroenterology* **102**, 720-727.

Chandranath, S. I., Bastaki, S. M. and Singh, J. (2002). A comparative study on the activity of lansoprazol, omeprazol and PD-136450 on acidified EtOH and - indomethacin-induced gastritis in the rat. *Clin. Exp. Pharmacol. Physiol.* **29**, 173-180.

Choi, C. H., Cha, Y. J., An, C. S., Kim, K. J., Kim, K. C., Moon, S. P., Lee, Z. H. and Min, Y. D. (2004). Molecular mechanisms of heptaplatin effective against cisplatin-resistant cancer cell lines: less involvement of metallothionein. *Cancer Cell Int.* **4**, 6-17.

Kasuya, Y., Urushidani, T. and Okabe, S. (1979). Effects of various drugs and vagotomy on indomethacin-induced gastric ulcers in the rat. *Jpn. J. Pharmacol.* **29**, 670-673.

Kim, J. M., Shin, J. E., Han, M. J., Baek, N. I. and Kim, D. H. (2003). Inhibitory effect of ginseng polyacetylenes on infection and vacuolation of *Helicobacter pylori. Natural Product Science* **9**, 158-160.

Koleva, I. I., Beek, T. A., Linssen, J. P. H., Groot, A. and Evastatieva, L. N. (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem. Anal.* **13**, 8-17.

Lee, E. J., Kim, K. S., Jung, H. Y., Kim, D. H. and Jang, H. D. (2005). Antioxidant activities of garlic (Allium sativum L.) with growing districts. *Food Sci. Biotechnol.* **14**, 123-130.

Leirisalo-Repo, M., Paimela, L., Koskimies, S. and Repo, H. (1993). Functions of polymorphonuclear leukocytes in early rheumatoid arthritis. *Inflammation* **17**, 427-442.

Leunk, R. D., Johnson, P. T., David, B. C., Kraft, W. G. and Morgan, D. R. (1988). Cytotoxic activity in broth-culture filtrates of Campylobacter pylori. *J. Med. Microbiol.* **26**, 93-

99.

Megraud, F. and Lamouliatte, H. (1992). *Helicobacter pylori* and duodenal ulcer. Evidence suggesting causation. *Dig. Dis. Sci.* **37**,769-772.

Mizui, T. and Dodeuchi, M. (1983). Effect of polyamines on acidified EtOH induced gastric lesion in rats. *Jpn. J. Pharmacol.* **33**, 939-945.

NIH Consensus Conference. (1994). *Helicobacter pylori* in peptic ulcer disease. NIH consensus development panel on *Helicobacter pylori* in peptic ulcer disease. *JAMA* **272**, 65-69.

Oyaizu, M. (1986). Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr.* **44**, 307-315.

Parsonnet, J. G., Friedman, D., Vandersteen, D. P., Chang, Y., Vogelman, J. H. and Sibley, R. K. (1991). *Helicobacter pylori* infection and the risk of gastric carcinoma. *N. Engl. J. Med.* **325**, 1127-1131.

Robert, A., Zamis, J. E., Lan caster, C. and Hancher, A. J. (1979). Cytoprotection by prostaglandins in rats. *Gastro-enterology* **77**, 433-443.

Shay, H., Komarrov, S. A., Fels, S. S., Meranze, D., Gruenstein, M. and Siplet, H. (1945). A simple-method for the uniform production of gastric ulceration in the rat. *Gastroenterol.* **4**, 43-51.

Uchida, M., Kawano, O., Misaki, N., Saitoh, K., and Irino, O. (1989). Relapse of acetic acid-induced gastric ulcer and gastric mucosal prostaglandin I_2 level in rats. *Chem. Pharmcol. Bull.* **37**, 805-807.

Yoshida, N., Yoshikawa, T., Iinuma, S., Arai, M., Takenaka, S., Sakamoto, K., Miyajima, T., Nakamura, Y., Yagi, N., Naito, Y., Mukai, F. and Kondo, M. (1996). Rebamipide protects against activation of neutrophils by *Helicobacter pylori. Dig. Dis. Sci.* **41**, 1139-1144.