— Research Letter —

Protective Effect of *Cimicifuga heracleifolia* Ethanol Extract and Its Constituents against Gastric Injury

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***Cimicifuga heracleifolia* (CH) ethanol extract and its constituents such as ferulic acid, caffeic acid, 24-epi-7,8-didehydrocimigenol-3-xyloside, and 23-O-acetylshengmanol 3-xyloside were investigated for their abilities to prevent gastric injury. To elucidate their gastric-protective effects, we assessed 1,1-diphenyl-2-picrylhydrazyl radical-radical scavenging activity and inhibition of *Helicobacter pylori* (*H. pylori*) and gastric cancer cells. Ferulic acid and caffeic acid exhibited higher free radical scavenging activity than other constituents and inhibited the colonization of *H. pylori* effectively. Furthermore, 24-epi-7,8-didehydrocimigenol-3-xyloside and 23-O-acetylshengmanol-3-xyloside showed cytotoxicity in gastric cancer cells (SNU638 and AGS cells). These results suggest the novel activities of CH ethanol extract and its constituents. CH ethanol extract and its constituents could be utilized for the treatment and/or prevention of gastric injury.**

Key words —— *Cimicifuga heracleifolia*, anti-helicobacter pylori, gastric protection, ferulic acid, caffeic acid

INTRODUCTION

Gastritis is an inflammation of the stomach lining and has various causes, involving contributions from aggressive (*e.g.*, gastric acid) and defective (*e.g.*, impaired mucus and bicarbonate production) factors. One factor is defective radical scavenging activity. A reduction in reactive oxygen species (ROS) protects against gastritis.[1)] One of the aggressive factors is infection by *Helicobacter pylori* (*H. pylori*), which is also a cause of gastric cancer.[2, 3)] Gastric cancer is a known cause of death from malignant disease.[4)] Gastric mucosa infected with *H. pylori* exhibit increases in ROS, which induce DNA damage.[5)] Eradication of *H. pylori* prevents gastritis, as well as gastric cancer. For example, antioxidant compounds such as ascorbic acid play important roles in terminating the development of gastritis and gastric cancer.[5–7)]

Rhizomes of the Asian species *Cimicifuga simplex*, *Cimicifuga dahurica*, *Cimicifuga foetida*, and *Cimicifuga heracleifolia* have been used as anti-inflammatory, antipyretic, and analgesic agents in traditional Japanese and Chinese medicine.[8)] The anti-inflammatory activity of *Cimicifuga heracleifolia* (CH) is greater than that of *Cimicifuga dahurica*.[9)] Chemical investigations of Cimicifuga species have led to the isolation of 9,19-cyclolanostane-type triterpenoids, fukiic acid esters, piscidic acid esters, caffeic acid derivatives, phenolic acid derivatives, and chromones.[10)] Ferulic acid and caffeic acid, the common constituents of Cimicifuga species, have the inhibitory effect of interleukin-8.[11, 12)] Triterpenoid glycoside (24-epi-7,8-didehydrocimigenol-3-xyloside and 23-O-acetylshengmanol-3-xyloside) is reported to have weak antibacterial activities.[13)] However, the anti-gastritis activities of CH and its constituents have not been revealed obviously.

In this study, we investigated the anti-gastritis activities of CH ethanol extracts and constituents by measuring their anti-oxidant activities, anti-*H. pylori* activities, and the cytotoxicity to the gastric cancer cells.

MATERIALS AND METHODS

Preparation of Plant Extract —— The rhizome of CH was purchased from Kyungdong Herbal market in Seoul, Korea. The roots were identified by

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Prof. K.H. Son, Andong National University, Korea. CH and its constituents were deposited as a voucher specimen at the Pharmaceutical Institute, College of Pharmacy, Duksung Women's University in Seoul, Korea. CH was extracted with 70% ethanol (in water) for 24 hr at room temperature. The extract was filtered by Whatman paper (No. 1) and concentrated by evaporator under reduced pressure.

Antioxidant Activity —— One milliliter of 1,1-diphenyl-2-picrylhydrazyl (DPPH, 150 μM) in methanol was added to 4 ml of extracts/compounds, and the mixture was stirred. After 30 minutes incubation at room temperature, the absorbance of the mixture was read against a blank at 520 nm. Scavenging DPPH free radical as a percentage (I%) was calculated as follows:

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where A_{blank} is the absorbance of the mixture without samples, and A_{sample} is the absorbance of the mixture with samples. The concentration providing 50% inhibition (IC_{50}) was determined from a plot of the percent inhibition versus concentration of the test agent. Ascorbic acid was used as a positive control.

Anti-*H. pylori* Activity —— The *H. pylori* strain HP43504 was obtained from the American Tissue Culture Collection (ATCC, Rockville, MD, U.S.A.). The inhibitory effects of CH ethanol extract and its constituents on the growth of *H. pylori* were investigated. Six hundred microliters of samples were mixed in a petri dish with 5.4 ml Brucella agar medium containing 7% horse serum. *H. pylori* [5×10^5 colony forming unit (CFU)] was seeded in this medium and placed for 3 days in an incubator at 37°C (AnaeroPak Campylo: 85% N_2, 10% CO_2, 5% O_2). *H. pylori* viability was determined by colony counts after 3 days incubation. Ampicillin was used as a positive control.

Cytotoxicity Assay —— AGS and SNU638 human gastric cancer cell lines were obtained from the Korean Cell Line Bank (Seoul, Korea). Gastric cancer cells were maintained at 37°C in minimum essential medium (MEM) containing 10% fetal bovine serum (FBS), 200000 IU/l penicillin, 200 mg/l of streptomycin, and 1 mM sodium pyruvate in a humidified atmosphere of 5% CO_2. Cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. The cells were seeded in 24-well plates at a density of 5×10^4 cell/well. After 24 hr incubation with sample, 100 μM MTT (5 μg/l in H_2O) was added to every well and the plates were incubated for 4 hr. Two hundred microliters dimethyl sulfoxide (DMSO) were added to each well and mixed by pipetting to dissolve MTT formazan completely. The relative cell viability was obtained by measuring absorbance using an ELISA reader (Molecular Devices, Menlo Park, CA, U.S.A.) at 540 nm.

RESULTS AND DISCUSSION

Antioxidant Activities of CH Ethanol Extract and Its Constituents

ROS, one of the aggressive factors, leads to acute and chronic inflammation in stomach.[14] Antioxidant action inhibits the involvement of ROS, disrupting the mechanism underlying several gastric disorders. The antioxidant activity of ascorbic acid plays a role in reducing gastric cancer, and therefore ascorbic acid was used as the positive control in tests of the actions of CH ethanol extract and its constituents. To determine antioxidant activity, DPPH radical scavenging activities were performed (Table 1). The hydrogen-donating activity of CH ethanol extract to the DPPH radical showed an IC_{50} = 15.58 μg/ml. Furthermore, ferulic acid (5.68 μg/ml) and caffeic acid (2.63 μg/ml) were more potent than CH ethanol extract. The other constituents exhibited low antioxidant activities with $IC_{50} > 300$ μg/ml. These results indicate that CH ethanol extract, ferulic acid and caffeic acid have significant free radical scavenging activity and are in accord with previous result.[15] Antioxidant activity of CH ethanol extract and its constituents represented the suppression of aggressive factors, suggesting the protection of gastric damage.

Anti-*H. pylori* Effect of CH Ethanol Extract and Its Constituents

H. pylori increases ROS,[5,16] and *H. pylori* in-

Table 1. DPPH Free Radical Scavenging Activity of CH Ethanol Extract and Its Constituents

Material	IC_{50} (μg/ml) of DPPH
CH ethanol extract	15.58
Ferulic acid	5.68
Caffeic acid	2.63
24-epi-7,8-didehydro-cimigenol-3-xyloside	> 300
23-O-acetylsheng-manol-3-xyloside	> 300
Ascorbic acid	< 1

Table 2. Inhibitory Activity of CH Ethanol Extract and Its Constituents on the Colonization of *H. pylori*

Material	Dose (μg/ml)	Colonization
Control	—	++++
CH ethanol extract	10	+++
	50	++
	100	—
Caffeic acid	2	++
	10	++
	20	—
Feruric acid	2	++
	10	—
	20	—
24-epi-7,8-didehydro-cimigenol-3-xyloside	15	++++
	75	+++
	150	—
23-O-acetylshengmanol -3-xyloside	10	++++
	50	+++
	100	—
Ampicillin	1	++
	10	—

+++, colonies ($4–5 \times 10^5$ CFU); ++, colonies ($2–4 \times 10^5$ CFU); +, colonies ($0–2 \times 10^5$ CFU); —, none.

fection is the major cause of gastritis and gastric cancer. To evaluate the inhibitory effect of CH ethanol extract and its constituents on *H. pylori*, the colonization of *H. pylori* was investigated. These showed dose-dependent anti-*H. pylori* effect (Table 2). Moreover, CH ethanol extract completely inhibited the colonization of *H. pylori* at 100 μg/ml.

In addition, CH constituents significantly inhibited the formation of *H. pylori* colonies. The inhibitory effect of ferulic acid was particularly high (10 μg/ml), equivalent to that of the positive control, ampicillin (10 μg/ml). 24-Epi-7,8-didehydrocimigenol-3-xyloside and 23-O-acetylshengmanol-3-xyloside also showed the anti-*H. pylori* activity (150 μg/ml and 100 μg/ml).

Gastric cancer is the second highest cause of cancer-related death in men and the fourth among women.[17] Various antibiotics have been used in attempts to eradicate *H. pylori* and prevent the associated gastritis and gastric cancer, but the efficacy of antibiotics is often reduced by drug-resistant bacteria. Recently, natural products such as cinnamic acid, courmarins, gallic acid, and their components have been reported to have anti-*H. pylori* activity.[18, 19] In our results, CH ethanol extract and its constituents were also cytotoxic for *H. pylori*, indicating the potential to decrease the risk of pathogen-derived gastritis and inhibit developing gastric cancer.

Table 3. Cytotoxicity of CH Ethanol Extract and Its Constituents

Material	IC_{50} (μg/ml)	
	SNU638	AGS
CH ethanol extract	> 200	> 200
Feruic acid	> 35	> 35
Caffeic acid	> 36	23.30
24-epi-7,8-didehydro-cimigenol-3-xyloside	49.76	77.22
23-O-acetylshengmanol -3-xyloside	31.86	59.33

Cytotoxic Effects of CH Ethanol Extract and Its Constituents on Gastric Cancer Cells

The cytotoxic effects of CH ethanol extract and its constituents investigated in SNU638 and AGS gastric cancer cell lines (Table 3). The CH constituents, 24-epi-7,8-didehydrocimigenol-3-xyloside and 23-O-acetylshengmanol-3-xyloside, exhibited greater cytotoxicity in SNU638 cells (IC_{50} = 49.76 and 31.86 μg/ml, respectively) than in AGS cells (IC_{50} = 77.22 and 59.33 μg/ml, respectively). However, the CH ethanol extract itself, feruic acid, caffeic acid had a slight cytotoxic effect and their IC_{50} couldn't be calculated in SNU638 cells within the range of the experimental concentration (> 200 μM, indicating > 35 or > 36 μg/ml in Table 3). Also, the cytotoxicity of CH ethanol extract and feruic acid in AGS cells was identical to that in SNU638 cells. The caffeic acid was more sensitive in AGS cells than in SNU638 cells, the IC_{50} of caffeic acid was 23.30 μg/ml in AGS cells. These results indicated that 24-epi-7,8-didehydrocimigenol-3-xyloside, 23-O-acetylshengmanol-3-xyloside and caffeic acid showed direct anti-cancer effects as well as protective effects against gastric injury induced by *H. pylori*.

The findings of this study support the conclusion that CH ethanol extracts and its constituents protect against potential gastric diseases as a result of free radical scavenging and anti-*H. pylori* activity.

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