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*), which is also a cause of gastric cancer. For example, antioxidant compounds such as ascorbic acid play important roles in terminating the development of gastritis and gastric cancer.

**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, Dukssung 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:

$$I\% = \left[ \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right] \times 100$$

where $A_{\text{blank}}$ is the absorbance of the mixture without samples, and $A_{\text{sample}}$ is the absorbance of the mixture with samples. The concentration providing 50% inhibition (IC50) 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 Campyo: 85% N₂, 10% CO₂, 5% O₂). 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% CO₂. 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 H₂O) 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. 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 IC50 = 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 IC50 > 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. 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, and H. pylori in-
Table 2. Inhibitory Activity of CH Ethanol Extract and Its Constituents on the Colonization of *H. pylori*

<table>
<thead>
<tr>
<th>Material</th>
<th>Dose (µg/ml)</th>
<th>Colonization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>++++</td>
</tr>
<tr>
<td>CH ethanol extract</td>
<td>10</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>2</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>—</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>2</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>—</td>
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<tr>
<td></td>
<td>20</td>
<td>—</td>
</tr>
<tr>
<td>24-epi-7,8-didehydro-cimigenol-3-xyloside</td>
<td>15</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>—</td>
</tr>
<tr>
<td>23-O-acetylshengmanol-3-xyloside</td>
<td>10</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>—</td>
</tr>
</tbody>
</table>

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

Infection 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. 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, coumarins, gallic acid, and their components have been reported to have anti-*H. pylori* activity. 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.

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

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, ferulic 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 ferulic 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.

### Acknowledgements

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