



## Inhibitory Activities of Palmatine from *Coptis chinensis* Against *Helicobacter pylori* and Gastric Damage

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*Helicobacter pylori* (*H. pylori*) is the most important factor of gastric disease in clinical practice. Moreover, smoking, stress and a poor diet may be additive factors for gastric damage. With these factors, increasing infection of *H. pylori* triggers gastritis, gastric ulcers and gastric cancer. To develop a new protective agent, we are concerned with plant-derived extract. The extract of *Coptis chinensis* (*C. chinensis*) and its constituents were investigated to assess their protective activities against gastric damage. The *C. chinensis* extract showed a scavenging effect against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide radicals, inhibition of *H. pylori* colonization and antiulcerogenic activities in rat. In particular, palmatine derived from *C. chinensis* was found to be the novel protective agent. It is better than the *C. chinensis* extract, berberine, a well-known constituent of *C. chinensis*. We suggest that palmatine from the root cortex of *C. chinensis* may be a good candidate for the development of new pharmaceuticals to prevent gastric disease.

**Key words:** *Coptis Chinensis*, *Helicobacter pylori*, Gastric damage, Palmatine

### INTRODUCTION

Stomach diseases including gastritis, gastric ulcers, and gastric cancer are chronic disorders and cause suffering for many people. These conditions are caused by an imbalance between aggressive factors and protective factors, infection with *Helicobacter pylori* (*H. pylori*) and lifestyle (nutritional deficiencies, smoking, excess alcohol consumption, and stress) (1). *H. pylori* is a microaerophilic bacterium found in the stomach. The infection rate worldwide is more than 50%, but the infection is usually asymptomatic (2). Since *H. pylori* is known as a trigger for gastritis and gastric cancer, many researchers are concerned with eliminating or attenuating *H. pylori*. Antibiotics and proton pump inhibitors or bismuth are used to treat and eradicate *H. pylori* (3). However, a great proportion of infected individuals are young in age and reinfection and antimicrobial resis-

tance are a problem for treatment. In this regard, plant-derived extracts are good candidates for prophylactic agents or medicines for stomach disease. Traditional herbal medicine has been used easily, and has broad-spectrum activities because plant-derived extracts are complex compound.

*Coptis chinensis* (*C. chinensis*), a member of Ranunculaceae family, has been utilized in traditional herbal medicine. The rhizome of *C. chinensis* possesses various pharmacological properties, such as antibacterial (4), antioxidative (5,6), anti-inflammatory (7,8) and anti-diabetes mellitus (9). We focused on these effects of *C. chinensis* and examined the role of *C. chinensis* and its constituents (palmatine and berberine) in gastric damage and *H. pylori* infection.

In this study, we elucidated the effect of *C. chinensis* and its constituents on stomach disease via antioxidant activity, colony formation of *H. pylori* and HCl/ethanol-induced gastric ulcer models. Finally, we characterized the novel pharmacological active material of *C. chinensis*.

### MATERIALS AND METHODS

**Preparation of the plant extract.** The root cortex of *Coptis chinensis* (Huang-lian, voucher specimen No. 2006042) and its constituents were provided from Prof. J. S. Choi (Pukyong National University, Korea), who identified the specimen (9). The samples were dissolved at the

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maximal concentration in distilled water and used with serial dilution as soon as possible.

**Antioxidant activity.** Scavenging of DPPH free radical, nitric oxide radical, and superoxide anion radical were determined, according to a previous study (10). Ascorbic acid was used as a positive control.

**Anti-*H. pylori* activity.** *H. pylori* strain (ATCC 43504) was obtained from ATCC (Rockville, MD, USA). Anti-*H. pylori* activity was examined according to a previous study (11). Briefly, brucella agar medium containing 7% horse serum (7 ml) was added to each sample (1 ml). *H. pylori* ( $5 \times 10^5$  CFU) was seeded in the sample containing media and then incubated for 3 days in a 37°C incubator using an anaerobic culture pack (AnaeroPak Campylo: 85% N<sub>2</sub>, 10% CO<sub>2</sub>, 5% O<sub>2</sub>). Viability of *H. pylori* was determined by colony-counts. Ampicillin was used as a positive control.

**Acid-neutralizing capacity (ANC).** Each sample (1 g) was added to 100 ml of 0.05 M HCl and then incubated for 1 hr at 37°C with shaking. ANC was determined by titrating with 0.1 M NaOH using methyl orange as an indicator. Hydrotalcite was used as a positive control.

**Animals.** Sprague-Dawley rats (male, weighing 180~200 g) were purchased from Samyook Animal Laboratories (Kyunggi-do, Korea) and were acclimatized to standard laboratory conditions ( $24 \pm 2^\circ\text{C}$ ,  $55 \pm 5\%$  humidity and 12 hr light/dark cycle) for 14 days in an animal facility at Dulsung Women's University. The experimental procedures for rats were conducted in accordance with the Guidelines of the Care and Use of Laboratory Animals, Dulsung Women's University. The animals were allowed free access to food (standard pellet diet) and water *ad libitum*.

**HCl/ethanol-induced mucosal membrane lesion.** Each sample was orally administered to the rats. After 30 min, 1 ml of HCl/ethanol solution (60% ethanol in 150 mM HCl) was administered orally for the induction of gastric lesions. The rats fasted for 1 hr, and were then anesthetized with ether. Their stomachs were then isolated and fixed in 2% formalin for 30 min. HCl-induced gastric damage was observed in the gastric mucosa as elongated black-red lines parallel to the long axis of the stomach of the rat. The total length (mm) of each lesion was determined. The lesion index was based on the average erosion length per rat. Cimetidine was used as a positive control.

## RESULTS AND DISCUSSION

Various radical oxygen species generate cell damage and can induce gastric damage (12). Antioxidant activity protects the stomach from radical oxygen species. *C. chinensis*

**Table 1.** Antioxidant effect of *C. chinensis* extract and its constituents

Material	IC <sub>50</sub> (µg/ml)		
	DPPH	NO	Superoxide radical
<i>C. chinensis</i> extract	35.44	< 9	19.11
Palmatine	> 300	23.24	27.00
Berberine	> 300	184.33	22.16
Ascorbic acid	< 1	< 1	< 1

extract and its constituents were evaluated for a radical scavenging effect. As shown in Table 1, the antioxidant effect of *C. chinensis* extract was the most potent. Palmatine and berberine showed a weak scavenging effect. The IC<sub>50</sub> of the DPPH-scavenging effect by palmatine and berberine could not be calculated using the maximum treated concentration. Because *C. chinensis* extract contains several active components, *C. chinensis* may give rise to the potent antioxidant effect, rather than palmatine and berberine. This result supports the conclusion that *C. chinensis* possesses an antioxidant effect, in accordance with several reports (13-15).

*H. pylori* is well-known as an inducing factor of gastritis, gastric ulcers and gastric cancer. Because blocking *H. pylori* activity protects against gastric damage, we investigated the antimicrobial activity of *C. chinensis* extract and its constituents against *H. pylori* (Table 2). *C. chinensis* extract (100 µg/ml) completely inhibited the colonization of *H. pylori*. We found that the novel constituent containing anti-*H. pylori* activity is palmatine. In particular, the anti-*H. pylori* activity of palmatine and berberine (16 µg/ml) was similar to that of ampicillin (positive control). This data indicated that palmatine and berberine derived from *C. chinensis* play a major role in its antimicrobial activity against *H. pylori*.

**Table 2.** Antimicrobial activity of *C. chinensis* extract and its constituents against *H. pylori*

Material	Dose (µg/ml)	Colonization <sup>§</sup>
Control	-	++++
<i>C. chinensis</i> extract	10	+++
	50	++
	100	-
Palmatine	4	++
	16	-
	32	-
Berberine	4	++
	16	-
	32	-
Ampicillin	1	++
	10	-

<sup>§</sup>Colony count: +++,  $4 \sim 5 \times 10^5$  CFU; ++,  $2 \sim 4 \times 10^5$  CFU; +,  $0 \sim 2 \times 10^5$  CFU; -, none.

**Table 3.** Acid neutralizing capacity of *C. chinensis* extract and its constituents

Material	Volume of NaOH consumption (μl)	Inhibition (%)
Control	120.0 ± 1.00	-
<i>C. chinensis</i> extract	108.3 ± 2.89*	9.7
Palmatine	108.3 ± 1.53*	9.7
Berberine	111.7 ± 2.89	6.9
Hydrotalcite	10.0 ± 0.77**	91.7

Significant difference, \* $p < 0.05$ , \*\* $p < 0.001$ , compared to the control.

The anti-*H. pylori* activity of berberine coincided with another study that berberine originated from different herbal extracts has anti-*H. pylori* activity (16).

Gastric acid is an aggressive factor that causes gastric damage. A protective agent against gastric damage should decrease the aggressive factors as well as increase the inhibiting factors. The acid neutralizing capacity of *C. chinensis* extract and its constituents was determined. As shown in Table 3, *C. chinensis* extract and palmatine slightly decreased NaOH consumption by 9.7% compared with the control. Furthermore, the inhibitory effect of berberine was 6.9%.

*In vitro* assay results elucidated the protective activities of *C. chinensis* extract and its constituents. We investigated its constituents for the protection of gastric damage in animal models. Control group (HCl/ethanol administration for 1 hr) induced gastric lesions ( $87 \pm 13.51$  mm), but palmatine (100 mg/kg) pre-treated rats showed a 45.9% decrease in gastric lesion length (Table 4). Palmatine proved to be as protective as cimetidine (200 mg/kg), which is known as a general treatment for gastric damage. This suggests that palmatine is the good candidate for a protective drug for gastritis and gastric ulcers. The inhibitory effect of berberine was about 10% weaker than that of palmatine. The data coincide with Li's report that *C. chinensis* (120 mg/kg) protected from ethanol-induced gastric damage and its effect was greater than berberine (100 mg/kg) (17).

We evaluated the activities of *C. chinensis* extract and its

**Table 4.** Effect of active constituents on HCl/ethanol induced gastric lesions

Material	Dose (mg/kg)	Lesion index (mm)	Inhibition rate (%)
Control		87.0 ± 13.51	-
Palmatine	50	52.2 ± 11.25*	40.1
	100	47.1 ± 6.8**	45.9
Berberine	50	60.5 ± 13.49*	30.5
	100	56.8 ± 13.44*	34.8
Cimetidine	200	45.7 ± 0.30**	47.5

The values are mean ± SEM of 6 animals.

Significant difference, \* $p < 0.05$ , \*\* $p < 0.01$ , compared to the control.

constituents in various gastric damage models. Anti-*H. pylori* activity and antiulcerogenic activity were indicated. Most of all, the novel effect of palmatine was identified. In addition to berberine, the anti-*H. pylori* activity of palmatine elucidated the protective effect of *C. chinensis* on gastric damage. We suggest that palmatine derived from *C. chinensis* plays a major role in the protection and therapy of *H. pylori*-induced gastritis and gastric ulcer.

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