Anti-*Helicobacter pylori* and Antiulcerogenic Activities of the Root Cortex of *Paeonia suffruticosa*

Joohee Jung,^a Ki Hwan Bae,^b and Choon-Sik Jeong^{*,a}

^a College of Pharmacy, Duksung Women's University; 33, 144-gil, Samyang-ro, Dobong-gu, Seoul 132–714, Korea; and

^b College of Pharmacy, Chungnam National University; 99 Daehak-ro, Yuseong-gu, Daejeon 305–764, Korea.

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In this study, we evaluated the gastric protective activities of mokdanpi *in vitro*. Further, we used experimental ulcer models to identify the active ingredients of mokdanpi. As a preliminary evaluation of mokdanpi ethanolic extract and its ingredients, we assessed its radical scavenging activities. In addition, its antimicrobial activity against *Helicobacter pylori* (*H. pylori*) was investigated. The antiulcerogenic activity of the active ingredients was evaluated in pylorus-ligated rats, an HCl/ethanol-induced and an absolute ethanol-induced ulcer model. We confirmed the scavenging effect of the ethanolic extract of mokdanpi and its ingredients against 2,2-diphenyl-1-picrylhydrazyl, nitric oxide and superoxide radicals, and we demonstrated that mokdanpi could inhibit the colonization of *H. pylori*. In an HCl–ethanol-induced ulcer model, gallic acid and catechin (100 mg/kg) inhibited 40.6% and 41.7% of gastric lesions, respectively. Catechin (100 mg/kg) significantly reduced ($p < 0.05$) the gastric secretion induced by pylorus ligation in rats in comparison to the control group. Gallic acid (100 mg/kg) significantly increased ($p < 0.05$) the mucus contents in an ethanol-induced ulcer model. The antioxidant ingredients (catechin and gallic acid) present in mokdanpi play a major role in antiulcerogenic activity, and demonstrate novel activity against *H. pylori*.

Key words *Paeonia suffruticosa*; *Helicobacter pylori*; gastric ulcer; gastric cancer; catechin; gallic acid

A large portion of the world's population is afflicted with gastric diseases, such as gastric ulcer, gastritis, and gastric cancer. These diseases are induced by stress, smoking, infection by *Helicobacter pylori* (*H. pylori*), and nutritional deficiencies.¹⁾ Gastric ulcers result from an imbalance between aggressive factors (*i.e.*, gastric acid, pepsin, stimulation of the vagus nerves, secretion of gastrin, and an increased number of parietal cells) and protective factors (*i.e.*, bicarbonate ion, mucus productivity, mucus secretion, and prostaglandins).²⁾ In particular, frequent gastritis or gastric ulcer is chronic and leads to death. Another important risk factor is infection from *H. pylori*. Since reports indicate that *H. pylori*-induced gastritis leads to gastric cancer, the control of *H. pylori* in the stomach has become a target of treatment for gastric diseases. The antibodies for *H. pylori* are higher in patients with gastric cancer than in control groups.³⁾ For eradication of *H. pylori*, a combination therapy with a proton pump inhibitor (PPI) or bismuth and antibiotics has been used. Nevertheless, the infection rate remains high and new therapeutic alternatives are needed because of antimicrobial resistance. In this regard, plant-derived extracts have been considered in the development of antiulcerogenic drugs.

Mokdanpi, the root cortex of *Paeonia suffruticosa* ANDREWS (Paeonaceae), is an important crude drug with analgesic, sedative, and anti-inflammatory activities, and has been used as a remedy for cardiovascular extravasated blood and female genital diseases in traditional Oriental medicine.^{4,5)} Methanolic extract of mokdanpi prevents the attachment and penetration of herpes simplex virus⁶⁾ and inhibits enzymes crucial to the life cycle of the human immunodeficiency virus.⁷⁾ In addition, mokdanpi extract exerts several important biological functions, such as hyaluronidase inhibitory activity,⁸⁾ antimicrobial activity,⁹⁾ melanin synthesis inhibition in cultured B-16 mouse melanoma cells,¹⁰⁾ inhibition of blood platelet

aggregation and antifibrinolytic activity, oxygen radical scavenging activity, and inhibition of phenylhydroquinone-induced oxidative DNA cleavage.¹¹⁾ Through studies of the chemical components of the *Paeonia* cortex, many glycosides were isolated and characterized from the polar fraction of mokdanpi ethanol-extract.^{12–14)} In addition, its multiple constituents have demonstrated a radical scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals.^{13,15)} We focused on the activities of mokdanpi and investigated its potential use as an antiulcerogenic and anti-*H. pylori* drug.

In this report, we examined the evidence indicating that mokdanpi extract and its ingredients provide gastric protection against ulcer and gastritis. We anticipated that mokdanpi may be a good candidate for the development of new pharmaceuticals to treat or prevent gastric disease including gastritis, gastric ulcer and cancer.

MATERIALS AND METHODS

Preparation of Plant Extract Mokdanpi was purchased from Kyungdong Herbal market in Seoul, Korea. The roots were identified by Prof. K. W. Bae, College of Pharmacy, Chungnam University, Korea. The voucher specimen (CNU-772) was deposited at the herbarium of the College of Pharmacy, Chungnam National University, Daejeon, Korea. Mokdanpi (20.0 kg) was extracted with 70% ethanol and evaporated under reduced pressure. The ethanol-extract (3 kg, yield 15%) was separated with hexane (400 g) and butanol (600 g). Paeonol (32 g, yield 8%) was obtained from the fraction of hexane, and paeoniflorigenone (190 mg, yield 0.37%), gallic acid (2.6 g, yield 4.2%) and catechin (200 mg, yield 0.33%) were fractionated from butanol. These information were provided in supplemental information.

Antioxidant Activity Antioxidant activity was determined by scavenging of DPPH free radical, nitric oxide radical, and superoxide anion radical, according to a previous

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* To whom correspondence should be addressed. e-mail: choonsik@duksung.ac.kr

study.¹⁶⁾ Ascorbic acid was used as a positive control.

Anti-*H. pylori* Activity *H. pylori* strain (ATCC 43504) was obtained from ATCC (Rockville, MD, U.S.A.). One milliliter of each sample was mixed with 7 mL of brucella agar medium containing 7% horse serum in the petri dish. *H. pylori* (5×10^5 colony forming unit (CFU)) was seeded in these media and then incubated for 3 d in a 37°C incubator using an anaerobic culture pack (AnaeroPak Campylo: 85% N₂, 10% CO₂, 5% O₂). Viability of *H. pylori* was determined by colony-counts after a 3-d incubation. Ampicillin was used as a positive control.

Acid-Neutralizing Capacity (ANC) One gram of Mokdanpi ethanol extract and its constituents was added to 100 mL of 0.05 M HCl and then incubated for 1 h 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, weighing 180–200 g, were purchased from Samyook Animal Laboratories (Kyunggi-do, Korea) and were acclimatized to standard laboratory conditions (24±2°C, 55±5% humidity and 12-h light/dark cycle) for 14 d in an animal facility at 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 samples dissolved in saline were administered in a volume of 0.5 mL per 100 g body weight.

HCl/Ethanol-Induced Mucosal Membrane Lesion The rats that received gallic acid and catechin were examined according to previous methods.¹⁶⁾ Thirty minutes after treatment, 1 mL of HCl-ethanol solution was administered orally for the induction of gastric lesions. After 1 h of fasting, each animal was sacrificed and the stomach was isolated. 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 rats. The lesion index was based on the average erosion length per rat. Cimetidine was used as a positive control.

Gastric Secretion Rats received gallic acid and catechin intraduodenally, followed by 24 h of fasting with free access to water prior to the experiment. At 4 h after the pyloric ligation, the animals were sacrificed, and the contents of the stomach were collected and centrifuged at 1050×g for 10 min. The total volume of gastric juice and pH were measured, and an acid output (mEq/mL) was determined by titrating the gastric juice with 0.1 M NaOH using methyl orange as an indicator.

Mucus Secretion Gallic acid and catechin were administered orally to the rats fasted for 24 h. After 30 min, absolute ethanol was given orally to induce the gastric lesions. One hour later, the animals were sacrificed and the secreted mucus was assessed.¹⁶⁾

Statistical Analysis Statistical analysis was performed using the Student *t*-test. Results were expressed as mean values±standard error of the mean (S.E.M.). Results were considered significant if *p*<0.05.

RESULTS AND DISCUSSION

Free radicals play an important role in the pathogenesis of injury in the digestive system.¹⁷⁾ The toxicity of reactive oxygen species (ROS) generated in the stomach causes acute and

chronic inflammation and damage to gastric cells and tissue.¹⁸⁾ To prevent gastric disease, the scavenging of free radicals is crucial. To assess the antioxidant activity of mokdanpi extract, we investigated its effect on free radical scavenging to determine the dose-response relationship (Table 1).

Mokdanpi extract effectively scavenged DPPH, nitric oxide (NO), and superoxide anion radicals. Among its ingredients, gallic acid was identified as having the potent quenching activities for DPPH and superoxide radicals at low IC₅₀ concentration (<9 and 28.74 µg/mL, respectively). Moreover, catechin had antioxidant activities against DPPH and NO with IC₅₀ concentrations of <9 and 16.72 µg/mL, respectively. Unfortunately, mokdanpi-extract and its constituents were weaker free radical scavengers than ascorbic acid as positive control in these assays. However, gallic acid and catechin sufficiently bear candidates of free radical scavenger.

In addition to ROS, *H. pylori* is responsible for several gastrointestinal disorders including ulcer, mucosa-associated lymphoid tissue lymphoma (MALT), and gastric carcinoma.¹⁹⁾ Toxic substances secreted from *H. pylori* cause inflammation and damage to gastric epithelial cells. The acute and chronic inflammation and denaturation of epithelial cells of the mucous membrane resulted in the loss of mucus and gastric cell death. Thus, we investigated the anti-*H. pylori* activity of mokdanpi extract as well as its ingredients. As shown in Table 2, mokdanpi extract (100 µg/mL) completely inhibited the colonization of *H. pylori*, and the antimicrobial effect was equivalent to that of ampicillin (10 µg/mL). The ingredients of mokdanpi showed anti-*H. pylori* activity. Especially, gallic acid blocked the colonization of *H. pylori* at a lower concentration (8.5 µg/mL) than ampicillin.

Because *H. pylori* infection affects approximately half of the world's population, the eradication of *H. pylori* is important for the prevention of peptic ulcers and gastric cancers.²⁰⁾ Although a combination of several antibiotics and a proton pump inhibitor has been widely used to remedy *H. pylori* infection, the appearance of drug-resistant bacteria has created the need for novel therapeutic alternatives. Plants may be the ideal source of novel antibacterial compounds, and recently, phytochemicals from many natural plants have been reported to have high anti-*H. pylori* activity.^{21,22)} However, the active compounds still need to be isolated and characterized. Lately, Ngan *et al.* reported that *Paeonia lactiflora* root constituents showed growth-inhibiting and bactericidal of *H. pylori*.²³⁾ Although genus is different between this report and our results, paeonol as a common constituent was coincided with our result. In this study, mokdanpi extract and its ingre-

Table 1. Free Radical Scavenging Effect of Mokdanpi-Ethanol Extract and Its Constituents

Material	IC ₅₀ (µg/mL)		
	DPPH	NO	Superoxide radical
Mokdanpi-extract	257	13.67	29.70
Paeonol	>300	>300	>300
Paeoniflorigenone	>300	>300	>300
Gallic acid	<9	>300	28.74
Catechin	<9	16.72	>300
Ascorbic acid	<1	<1	<1

Table 2. Anti-*H. pylori* Activity of Mokdanpi Extract and Its Ingredients

Material	Dose ($\mu\text{g/mL}$)	Colonization
Control	—	++++
Mokdanpi extract	10	+++
	50	++
	100	—
Paeonol	1.66	++
	8.3	+
	16.6	—
Paeoniflorigenone	9.54	+++
	47.7	++
	95.4	—
Gallic acid	1.7	++
	8.5	—
	17	—
Catechin	2.9	++
	14.5	—
	29	—
Ampicillin	1	++
	10	—

+++ , colonies ($4\text{--}5\times 10^5$ CFU); ++ , colonies ($2\text{--}4\times 10^5$ CFU); + , colonies ($0\text{--}2\times 10^5$ CFU); — , none

Table 3. Acid-Neutralizing Capacity of Mokdanpi Extract and Its Ingredients

Material	NaOH consumption vol (μL)	Inhibition (%)
Control	120.0 ± 1.00	0
Mokdanpi extract	$106.7\pm 2.89^*$	11.1
Paeonol	120.0 ± 0.00	0
Paeoniflorigenone	$94.3\pm 4.04^*$	15.5
Gallic acid	$136.7\pm 5.77^*$	-13.9
Catechin	117.0 ± 1.73	2.5
Hydrotalcite	$10.0\pm 0.77^{**}$	91.7

The values are mean $\pm$ S.E.M. of 3 experiments. Significant difference *, $p<0.05$; **, $p<0.001$ compared to the control group

dients interrupted the proliferation of *H. pylori*. The anti-*H. pylori* activity is a newly identified gastric protective function of mokdanpi extract.

Another attack factor for gastric ulcer is gastric acid. The neutralization of gastric luminal acid is very effective in healing a gastric ulcer.²⁴⁾ To investigate the acid-neutralizing capacity (ANC), NaOH consumption was compared between control and mokdanpi extract. As shown in Table 3, mokdanpi extract decreased NaOH consumption by 11.1% as compared to a control. Paeoniflorigenone also showed a 15.5% decrease in NaOH consumption, whereas paeonol and catechin did not show inhibitory action. These ANC was weaker than that of hydrotalcite as positive control. Additionally, subacid gallic acid increased NaOH consumption in comparison to the control. However, mokdanpi extract contained various components that protected against gastric damage from secreted gastric acid. The ANC of mokdanpi extract is supposed to increase the pH of the gastric content. In the *in vitro* assays, the data indicate that mokdanpi extract and several ingredients can protect against gastric damage from ROS, *H. pylori* infection and gastric acid. We further studied active ingredients

Table 4. Effect of Ingredients from Mokdanpi on HCl-Ethanol Induced Gastric Lesion

Material	Dose (mg/kg)	Lesion index (mm)	Inhibition rate (%)
Control		87.0 ± 13.51	—
Gallic acid	50	79.5 ± 7.09	8.6
	100	$51.7\pm 9.01^*$	40.6
Catechin	50	70.3 ± 18.01	19.2
	100	$50.75\pm 6.60^*$	41.7
Cimetidine	200	$45.7\pm 0.30^{**}$	47.5

The values are mean $\pm$ S.E.M. of 6 animals. Significant difference *, $p<0.05$; **, $p<0.01$ compared to the control group.

Table 5. Effect of Ingredients from Mokdanpi on Gastric Secretion in Pylorus-Ligated Rats

Material	Dose (mg/kg)	Volume (mL)	pH	Total acid output (mEq/4h)
Control		4.2 ± 1.2	1.38 ± 0.8	0.38 ± 0.16
Gallic acid	100	4.1 ± 1.8	0.90 ± 0.3	0.37 ± 0.12
Catechin	100	$3.1\pm 1.1^*$	$2.60\pm 1.9^*$	$0.25\pm 0.17^*$
Cimetidine	250	$1.7\pm 0.5^{***}$	$3.50\pm 0.8^{**}$	$0.22\pm 0.13^*$

Total gastric juice volume and pH were measured at 4h after the pyloric ligation. The values are mean $\pm$ S.E.M. of 6 animals. Significant difference *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ compared to the control group

(gallic acid and catechin) that prevent gastric ulcer in *in vivo* rat models.

The intragastric administration of HCl-ethanol (60% in 150mm HCl) to rats made the gastric tissues thinner and fainter, and created multiple band-like lesions in the gastric mucosa, with 87.0 ± 13.51 mm of the lesion index, whereas normal mice did not show any gastric lesions (data not shown). The oral administration of gallic acid and catechin significantly reduced the HCl-induced gastric lesions in rats (Table 4). Gallic acid and catechin dose-dependently reduced these lesions by inhibition of HCl as the attack factor. Gallic acid (100mg/kg) and catechin (100mg/kg) inhibited approximately 40.6 and 41.7% of HCl-ethanol-induced gastric lesions, respectively, and these results were similar to those for cimetidine (200mg/kg), a positive control (approximately 47.5% inhibition). The effect of gallic acid and catechin was remarkable at the lower concentration than that of cimetidine. In a pylorus-ligated rat model, gastric juice was compared between the control group and active ingredient-treated group. Gastric volume, pH, and acid output were measured and the results are shown in Table 5. The gallic acid-treated group did not show a significant difference in comparison to the control group. However, the amount of gastric secretion by catechin was 3.1mL, which was less than that of control (4.2mL). Catechin increased pH to 2.6 in gastric juice, as compared to control (pH 1.38), and showed a decline in the total acid output to 0.25mEq/4h as compared to a control (0.38mEq/4h). The data indicated that catechin decreased acid secretion. Unfortunately, gallic acid and catechin were slightly weaker than cimetidine. Nevertheless, mokdanpi extract including gallic acid, catechin and *etc.* may be more effective. Taken together, it is expected that mokdanpi extract containing catechins will suppress the attack factor through the inhibition of total

Table 6. Effect of Ingredients from Mokdanpi on Mucus Contents from Absolute Ethanol-Induced Gastric Lesion in Rats

Material	Dose (mg/kg)	Mucus content (μg as alcian blue)
Control		173.9 $\pm$ 7.68
Gallic acid	100	217.3 $\pm$ 3.78**
Catechin	100	155.8 $\pm$ 7.24*
Sucralfate	375	160.4 $\pm$ 6.72*

Control means the absolute ethanol-induced rats orally. The values are mean $\pm$ S.E.M. of 6 animals. Significant difference *, $p<0.05$; **, $p<0.01$ compared to the control group.

acid output, thus playing an important role in the anti-gastric effect, including the reduction of the lesion index *in vivo*. In HCl-ethanol-induced and pylorus-ligated rat models, we evaluated the attenuation of attack factors by mokdanpi-derived ingredients. We also investigated the effect of mokdanpi-derived ingredients on protective factors by analyzing mucus secretion. Ethanol diminished the amount of mucus secretion in gastric epithelial cells. The absolute ethanol-treated group (as control) showed 173.9 μg of mucus content, but the gallic acid-treated group (100 mg/kg) had a significantly greater mucus content of 217.3 μg (Table 6). The catechin-treated group showed a decrease in mucus content as sucralfate. Gallic acid is believed to protect the stomach from attack factors by increasing mucus secretion, as well as by its anti-*H. pylori* activity and by its acid neutralizing capacity. The secretion of mucus in response to sucralfate, which is an effective anti-ulcer medicine, was lower than that of the control group. The reason might be that the protective action of sucralfate for stomach damage is mediated through sucralfate-coating, not by the increase of mucus secretion in response to ethanol irritation. Judging from the above results, the gastro-protective activity of gallic acid, a constituent of mokdanpi, is expected to be due to the stimulation of mucus secretion.

CONCLUSION

We identified anti-*H. pylori* activity of mokdanpi extract, suggesting that it could be used to protect against gastric ulcer as well as gastric cancer. The antiulcerogenic activity of mokdanpi was induced by the enhancement of protective factors such as antioxidant activity and potent ANC. The active ingredients of mokdanpi extract, such as gallic acid and catechin, showed protective effects on various gastric ulcer models. Thus, our data support a role for this traditional medicine in the protection and therapy of *H. pylori*-induced gastric ulcer and gastric cancer.

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