



Gastroprotective Activity of *Padina minor* Yamada

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ABSTRACT

Padina minor Yamada, a brown alga which is found to be abundant on both sides of the coastal area of the gulf of Thailand and the Andaman Sea was examined for gastroprotective activity. The aqueous extract of *P. minor* (Aq. *P*) given orally to rats at the doses of 100, 200 and 500 mg/kg significantly inhibited gastric ulcer formation induced by (1) restraint water immersion stress (2) acid ethanol (HCl /EtOH), (3) indomethacin and (4) histamine. In the pylorus-ligated rat experiment, the Aq. *P* caused a decrease of the total acidity and an increase in gastric pH. Additionally, the Aq. *P* could not preserve the mucus content in the gastric wall of rats with gastric ulceration induced by HCl /EtOH. Results were obtained from the isolated guinea-pig right atrium experiment of which the inhibitory effects of cimetidine (Histamine H₂-antagonist) and the Aq. *P* on histamine-induced chronotropic responses were determined and these results suggest that they share a similar mechanism. The findings therefore indicate that *P. minor* possesses a gastroprotective activity, which involves an anti-secretory mechanism mediated via histamine H₂-antagonism. The polysaccharide present in the *P. minor* is likely to contribute to the gastroprotective activity.

Keywords: anti-secretory activity, gastroprotective activity, H₂-antagonism, *Padina minor*, polysaccharide.

1. INTRODUCTION

Padina minor Yamada is a brown marine alga found in abundance on both sides of the coastal area of the gulf of Thailand and the Andaman Sea. Seaweeds (marine algae) are consumed in diet and as medicines in Asian countries. Traditional medicines in Asia have used marine algae for the treatment of cancer and to provide many health benefits [1, 2]. Marine algae have also proven to be rich

sources of structurally diverse bioactive compounds with valuable pharmaceutical and biomedical potential [3]. Recent research has pointed out that the consumption of brown marine algae could inhibit the occurrence of breast cancer, other inflammatory disorders and reduce cholesterol [4]. Sulfated polysaccharides from brown algae are reported to have blood anticoagulant, anti-tumour,

anti-mutagenic, anti-complementary, immunomodulating, hypoglycemic, antiviral, hypolipidemic and anti-inflammatory activities [5-8]. Additionally, the antioxidant activity of marine brown algae has been suggested to be due to their phenolic contents [9-10].

Gastric ulcer is a chronic disease and results from an imbalance between aggressive factors and defensive factors which maintain the mucosal integrity through the endogenous defense mechanisms [11]. Aggressive factors consist of acid and pepsin, *Helicobacter pylori* infection and nonsteroidal anti-inflammatory drugs (NSAIDs). Histamine is a critical regulator of acid production through the H₂ subtype of the receptor. Defensive factors consist of gastric mucus, gastric mucosal blood flow and a gastric mucosal barrier [12]. Anti-gastric ulcer activity of a brown alga, *Sargassum polycystum*, has been reported [13].

The aqueous extract of *Padina minor* Yamada (Aq. *P*) was found to show interesting pharmacological activities such as hypotensive [14] and antioxidant [15] properties. A preliminary study showed that the Aq. *P* could suppress the gastric ulceration induced by restraint water immersion stress-induced gastric ulcers. The present work was performed to evaluate the gastroprotective activity of Aq. *P* by employing various models of gastric ulceration in rats. Possible mechanism (s) involving in the activity were also examined.

2. MATERIALS AND METHODS

2.1 Extraction of *Padina minor*

Fresh brown marine alga, *Padina minor* Yamada (Division Phaeophyta), was collected from Naiyang Beach, Phuket Province, Thailand. The alga was greenish brown to yellowish brown in color, and growing on rock fragments or pebbles in intertidal areas. It appeared as small thallus, fan-shaped and 3-6 cm high. The blade was thin and composed of two layers. A voucher specimen

(no. 159) has been deposited at the herbarium of the Applied Algal Research Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

The collected alga was washed thoroughly in tap water and dried in an oven at 60°C for 48 h. The dried alga at a weight of 200 g was boiled with 1 L of distilled water at 60°C for 24 h and then filtered through 4 layers of gauze. The filtrate was evaporated *in vacuo* and lyophilized to obtain a dried aqueous extract of *P. minor* (Aq. *P*). The yield was 20.1%. The extract was dissolved in distilled water to the required concentrations before being used.

The crude polysaccharide was extracted from the Aq. *P* as follows [16]. Ten grams of the Aq. *P* was dissolved in 100 ml of deionized water, and ethanol was added slowly to a final concentration of 75% (v/v) while stirring. After standing overnight at 4°C, it was centrifuged at 10,000 rpm for 20 min and the precipitate was lyophilized to yield the crude polysaccharide (yield 3.29% of dried *P. minor*).

2.2 Gas Chromatography - Mass Spectrometry (GC-MS) Analysis

Chemicals analysis of the Aq. *P* was determined by GC-MS (a service provided by The Science and Technology Service Center, Chiang Mai University). The GC-MS instrument GC 6890 (Agilent Technologies Co., Ltd. USA.) was used, with a HP-5MS column (30m × 0.25mm i.d.), film thickness 0.25 μm, carrier gas Helium and injector temperatures 270 °C. The GC temperature program consisted of an initial temperature of 50°C; 10°C/min to reach a final temperature of 250°C. The analysis was carried out on the basis of MS spectra and results were compared with the database (Wiley 7n.l).

2.3 Laboratory Animals

Male Sprague-Dawley rats and guinea-pigs were obtained from the National Laboratory Animal Center, Salaya Mahidol University, Thailand. They were acclimatized for at least 7 days in an animal room where the temperature was maintained at $22 \pm 3^\circ\text{C}$ with a 12 h light-dark cycle. The animals had free access to water and a standard diet (Perfect Companion, Bangkok, Thailand). All animals received humane care in compliance with the ethics in the use of animals issued by the National Research Council of Thailand 1999, and the experimental procedures were approved by the Animal Ethics Committee, Faculty of Medicine, Chiang Mai University.

2.4 Experimental Models of Gastric Ulceration in Rats

Groups of 48 h fasted rats were given 3 doses of the Aq. *P*, cimetidine, and distilled water by intragastric administration. After sixty minutes, gastric ulcerations were induced in the rats.

2.4.1 Restraint Water Immersion Stress-induced Gastric Ulcers

The method of Takagi et al. [17] was used. Briefly, the rats were restrained individually in stainless steel cages and immersed up to their xiphoid in a water bath maintained at $22 \pm 2^\circ\text{C}$ for 5 h. Then the rats were sacrificed and examined for gastric ulcers.

2.4.2 HCl/EtOH-induced Gastric Ulcers

A mixture of 1.0 ml HCl/EtOH (60 ml EtOH + 1.7 ml HCl + 38.3 ml H₂O) was orally administered to the rats according to the method of Mizui and Doteuchi [18]. The rats were sacrificed and examined for gastric ulcers after 60 min.

2.4.3 Indomethacin-induced Gastric Ulcers

Indomethacin suspended in 0.5% carboxymethylcellulose was intraperitoneally injected at a dose of 30 mg/kg, [19]. After 5 h the rats were sacrificed and examined for gastric ulcers.

2.4.4 Histamine-induced Gastric Ulcers

Histamine was injected intraperitoneally at a dose of 10 mg/kg [20]. The rats were sacrificed and their stomachs were examined for gastric ulcers after 4 h.

2.5 Evaluation of the Gastric Ulcers

After each rat was sacrificed, the stomach was removed, opened along the greater curvature and the glandular portion of the stomach was examined. The length in mm of each lesion was measured under a dissecting microscope (10x). The sum of the length of all lesions was designated as the ulcer index.

2.6 Pylorus Ligation in Rats

Pylorus ligation as described by Shay et al. [21] was performed 1 h after oral administration of the Aq. *P* to the rats. Briefly, the rats were lightly anesthetized with ether. The abdomen was opened and the pylorus was ligated. The abdomen was closed by suturing. The animals were sacrificed 5 h later by an overdose of ether. The stomach was removed and its content was subjected to measurements of volume, pH and titratable acidity.

2.7 Gastric Wall Mucus of Rats with HCl/EtOH Induced Gastric Ulcers

The Aq. *P* was administered orally to 48 h fasted rats 60 min prior to induction of gastric ulcers by HCl/EtOH. Sixty minutes later, the rats were sacrificed and their stomachs were excised and opened along the lesser curvature. Gastric wall mucus was then measured by

Alcian Blue Method [22]. Briefly, the stomach was immersed in 0.1% w/v Alcian blue solution for 2 h. The excessive dye was then removed by two successive rinses in 0.25 M sucrose solution. Dye complex with gastric wall mucus was extracted with 0.5 M $MgCl_2$ for 2 h. The blue extract was then shaken vigorously with an equal volume of diethyl ether and the resulting emulsion was centrifuged. The optical density of Alcian blue in the aqueous layer was read against a buffer blank at 580 nm using a spectrophotometer. The quantity of alcian blue extract per gram of wet stomach was then calculated from a standard curve.

2.8 Isolated Guinea Pig Right Atria Experiment

Male Guinea pigs were sacrificed by a blow to the head. The right atria was dissected and then suspended in 20 ml organ bath containing Feigen's solution ($NaCl = 0.9$, $NaHCO_3 = 0.6$, $KCl = 0.42$, $CaCl_2 = 0.62$ and glucose = 1.0 g/L) with a control temperature of 37°C and was aerated continuously with 95% O_2 and 5% CO_2 gas mixture. The force and rate of contraction of the right atria were recorded via a force displacement transducer (FT03, Grass Instrument Co., U.S.A.) under a resting tension of 1 g and via a tachograph (Model 7DA; Grass Instrument Co., U.S.A.) respectively, and were displayed on a polygraph (Model 7D; Grass Instrument Co., U.S.A.) A 30 min equilibration period was allowed before starting the experiment. Histamine 10^{-5} M was used to induce an increased rate of atrial contraction. The Aq. P and cimetidine were tested and compared with the response in the experiment.

2.9 Statistical Analysis

The data were expressed as mean \pm S.E.M. Statistical comparison between groups

was analyzed by using one-way analysis of variance (ANOVA) and post-hoc least-significant difference (LSD) test. *p* values less than 0.05 were considered significant.

2.10 Drugs and Chemicals

Acetylcholine iodide, Histamine and Indomethacin were obtained from Sigma Chemical Company, St. Louis, U.S.A. Cimetidine was obtained from Siam Pharmaceutical Co., Ltd., Thailand. Alcian blue was obtained from Fluka Chemicals Co., Ltd., Japan. All other reagents used in this work were of analytical grade.

3. RESULTS

3.1 GC-MS Analysis of the Aq. P

Figure 1 illustrates the GC-MS spectra, and screening analysis of the Aq. P.

3.2 Effect of the Aq. P on Gastric Ulcer in Rats

As shown in Table 1, the Aq. P showed dose related gastroprotective activity when tested in various experiment models. The Aq. P at doses of 100, 200 and 500 mg/kg significantly inhibited gastric ulcer induced by indomethacin and histamine, whereas significant inhibition was seen with the doses of 200 and 500 mg/kg when tested in restraint water immersion stress and HCl/EtOH-induced gastric ulcer. The Aq. P showed marked effect in the histamine model. The effectiveness of gastroprotective activity of the Aq. P at the dose of 200 mg/kg was compared among the 4 models of gastric ulcer in rats. It was found that the activity of the Aq. P was 0.60, 0.93, 0.78, and 1.07 times of cimetidine at the dose of 100 mg/kg, when tested in restraint water immersion stress, HCl/EtOH, indomethacin and histamine models, respectively.

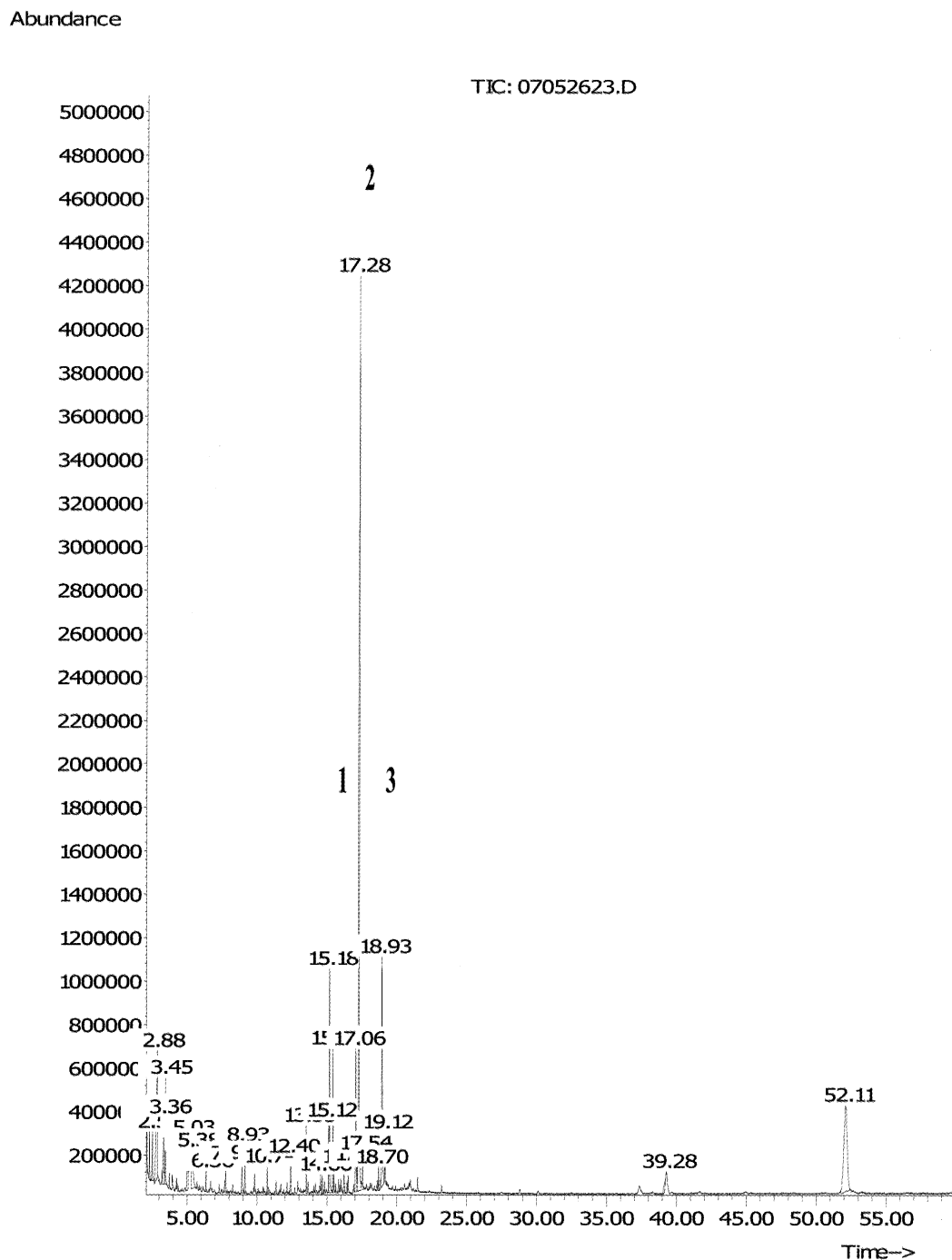


Figure 1. Gas chromatogram obtained for the aqueous extract of *Padina minor*.

1 = Myristinic acid

2 = Palmitic acid

3 = Oleic acid

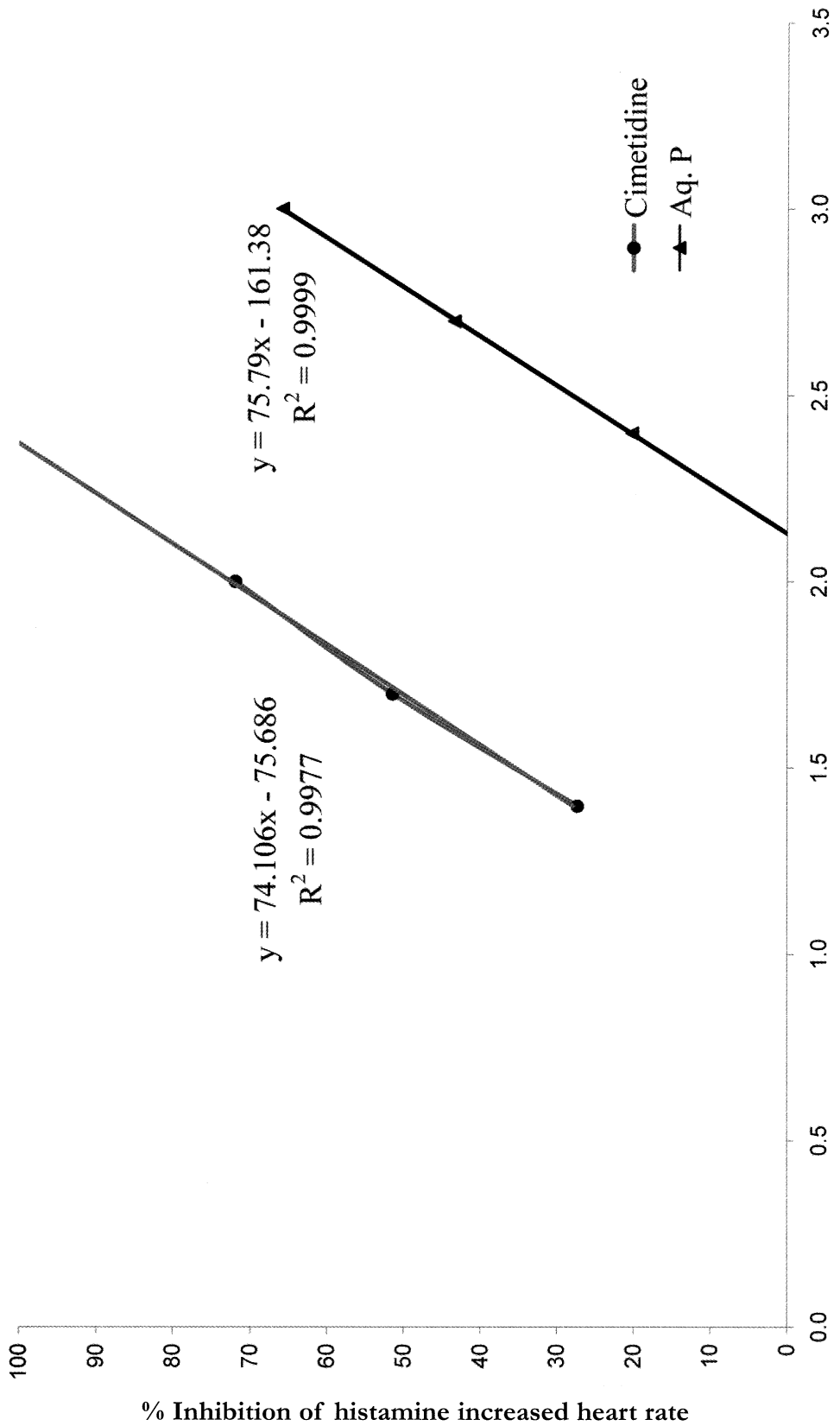


Figure 2. Regression lines of concentration-effect of cimetidine and aqueous extract of *Padina minor* (Aq. P) on histamine induced increased heart rate of isolated guinea-pig right atria.

Table 1. Effect of the aqueous extract of *Padina minor* on gastric ulcers induced in rats by restraint water immersion stress, HCl/ETOH, indomethacin and histamine.

Group	Gastric ulcer induced by									
	Restraint water immersion stress		HCl/EtOH		Indomethacin		Histamine			
	Ulcer index (mm)	Inhibition (%)	Ulcer index (mm)	Inhibition (%)	Ulcer index (mm)	Inhibition (%)	Ulcer index (mm)	Inhibition (%)		
Control	10.5 ± 1.2	-	104.6 ± 11.6	-	5.0 ± 1.0	-	10.03 ± 2.34	-		
Cimetidine 100 mg/kg	0.7 ± 0.3***	93.3	66.5 ± 15.3*	36.4	0.0 ± 0.0***	100	0.66 ± 0.30***	93		
<i>P. minor</i> 100 mg/kg	7.9 ± 0.7	24.8	80.1 ± 15.2	23.4	1.9 ± 0.6*	62	0.35 ± 0.25***	96		
200 mg/kg	4.6 ± 1.8***	56.2	69.3 ± 10.2*	33.7	1.1 ± 0.5**	78	0.10 ± 0.10***	99		
500 mg/kg	2.3 ± 0.7***	78.1	20.9 ± 7.5***	80.0	0.2 ± 0.1***	96	0.14 ± 0.14 ***	98		

Data expressed as mean ± S.E.M. (n = 8)

Significant difference from control group: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 2. Effect of crude polysaccharide of *Padina minor* on water immersion-induced stress ulcers in rats.

Group	Ulcer index (mm)	%Inhibition
Control (Distilled water)	14.8 ± 1.1	-
Cimetidine 100 mg/kg	0.7 ± 0.3**	95.3
Crude polysaccharide 100 mg/kg	11.4 ± 1.9*	23.0
200 mg/kg	7.9 ± 1.0***	46.6
500 mg/kg	1.5 ± 0.6***	89.9

Data expressed as mean ± S.E.M. (n=6)

Significant difference from control group: (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Table 3. Effect of the aqueous extract of *Padina minor* on gastric secretion in rats.

Group	Gastric vol. (ml)	Gastric pH	Acidity mEq/L
Control (5%Tween)	3.7 ± 0.4	1.8 ± 0.4	219.6 ± 15.4
Cimetidine 100 mg/kg	2.5 ± 0.2	7.3 ± 0.2***	121.0 ± 10.1***
<i>P. minor</i> 100 mg/kg	3.6 ± 0.7	3.0 ± 0.5*	170.8 ± 20.8*
200 mg/kg	4.7 ± 1.0	3.8 ± 0.4**	145.0 ± 18.4**
500 mg/kg	3.2 ± 0.7	4.0 ± 0.7**	144.0 ± 18.7**

Data expressed as mean ± S.E.M. (n = 6)

Significant difference from control group: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 4. Effect of the aqueous extract of *Padina minor* on gastric wall mucus content of rats with HCl/EtOH induced gastric ulcer.

Group	Gastric wall mucus (ug Alcian blue/g wet stomach)
Normal	322.6 ± 24.1
HCl/EtOH induced gastric ulcer Control	157.3 ± 7.3 [#]
Cimetidine 100 mg/kg	240.7 ± 21.9 ^{**}
<i>P. minor</i> 100 mg/kg	167.9 ± 11.0 [#]
200 mg/kg	165.9 ± 18.9 [#]
500 mg/kg	163.5 ± 12.7 [#]

Data expressed as mean ± S.E.M. (n = 6)

Significantly different from control group: * $p < 0.05$

Significantly different from normal group: [#] $p < 0.05$

3.3 Effect of the Crude Polysaccharide of *P. minor* on Gastric Ulcer induced by Restraint Water Immersion Stress in Rats

Table 2 shows the dose related inhibitory effect of the crude polysaccharide of *P. minor* on gastric ulcer induced by restraint water immersion stress.

3.4 Effect of the Aq. *P* on Gastric Acid Secretion

Cimetidine (100 mg/kg) and the Aq. *P* at the dose of 100, 200 and 500 mg/kg caused significantly decreased total gastric acidity and increased gastric pH (Table 3).

3.5 Effect of the Aq. *P* on Gastric Wall Mucus of HCl/EtOH Induced Gastric Ulcerated Rats

The gastric mucus content was significantly decreased in HCl/EtOH ulcerated rats ($p < 0.05$). Pretreatment with cimetidine effectively lessened the decrease of the gastric mucus content of the ulcerated rats, whereas pretreatment with the Aq. *P* could not protect against a loss of gastric mucus (Table 4).

3.6 Effect of the Aq. *P* on Histamine Induced Increased Heart Rate of Guinea Pig Atria

The Aq. *P* and cimetidine exhibited the inhibitory effect on the histamine-induced heart rate in a concentration-dependent manner (Figure 2). The IC₅₀ values of the Aq. *P* and cimetidine were found to be 446 and 0.431 $\mu\text{g/ml}$, respectively. The slope values of the regression lines of cimetidine (74.11), and the Aq. *P* (75.79) were practically equal ($p < 0.05$).

4. DISCUSSION

The Aq. *P* at the dose of 100, 200 and 500 mg/kg showed gastroprotective activity when tested in rats with gastric ulceration induced by (1) restraint water immersion stress

(2) EtOH/HCl (3) indomethacin and (4) histamine.

The restraint water immersion stress, EtOH/HCl, and indomethacin induced gastric ulcer models are among the most commonly utilized for evaluation of anti-ulcer activity in rats [23, 24]. The gastric lesions induced by the restraint water immersion stress, are suggested to be produced by an increase in gastric acid secretion [25], a decrease of the mucosal microcirculation [26] and a decrease in mucus content [27]. In the HCl/EtOH-induced gastric ulceration model, HCl causes severe damage to gastric mucosa [28] whereas ethanol produces necrotic lesions by direct necrotizing action which in turn reduces defensive factors such as the secretion of bicarbonate and production of mucus [29]. Indomethacin induces ulcer formation by depleting cytoprotective prostaglandins (e.g. PGE₂ and PGI₂), and by irritating the gastrointestinal mucosa [30].

The roles of reactive oxygen (ROS) species in lipid peroxidation have been suggested in the pathogenesis of experimental gastric lesions induced by stress, ethanol and by indomethacin [31-33]. Interestingly, the Aq. *P* was found to possess an antioxidant activity when tested in DPPH (1-diphenyl-2-picrylhydrazyl), ABTS⁺⁺ (2,2'-azino-bis 3-ethylbenzthiazoline -6-sulfonic acid) and lipid peroxidation assays, and was shown to contain phenolic substances [14]. GC-MS analysis of the Aq. *P* revealed the presence of substances e.g. palmitic and oleic acid which have been shown to have antioxidant activity [34, 35]. It is therefore possible that the anti-gastric ulcer activity of *P. minor* is mediated via its antioxidant activity.

Gastric-wall mucus plays an important role as a defensive factor against gastrointestinal damage [36]. However, the Aq. *P* failed to preserve the gastric mucus content of rats

with gastric ulcers induced by HCl/EtOH.

The Aq. *P.* showed a marked inhibitory effect on histamine induced gastric ulcers. Histamine produces gastric ulceration by enhancing gastric acid secretion via stimulation of histamine H₂-receptor [11]. H₂ antagonists inhibit gastric acid secretion (antisecretory activity) by competing with histamine at the H₂-receptor. Similarly to cimetidine (a H₂ antagonist), the Aq. *P.* showed antisecretory activity causing decreased gastric acidity, and increased gastric pH when tested in pylorus ligated rats. The Aq. *P.* was then explored for H₂-receptor antagonist activity by being tested against histamine induced increased heart rate (chronotropic effect) of the isolated guinea-pig atria [37]. Both cimetidine and the Aq. *P.* inhibited a histamine-induced chronotropic response, and their slope values from the regression lines of the concentration-effect were practically equal. Thus it is suggested that *P. minor* shares a similar mechanism as cimetidine, a histamine-H₂ antagonist.

Polysaccharides of marine algae have been found to have various biological activities [5, 6]. The present study has demonstrated the gastroprotective activity of the crude polysaccharides from the Aq. *P.* However, the amount of the crude polysaccharide in the Aq. *P.* was only 18.6%, thus suggesting that other constituents present in the Aq. *P.* may also play roles in the gastroprotective activity of *P. minor*. Among the polysaccharides of brown marine algae, alginate has the ability of forming viscous solutions and gels [38]. Thus, gastroprotective activity of *P. minor* may be mediated via its gel-formation property causing the adherence to the epithelial cells while protecting the gastric mucosa as it interacts with acid directly.

5. CONCLUSION

The present study has shown that *P. minor* exhibits gastroprotective activity which

involves the anti-secretory mechanism mediated via histamine H₂-antagonism. It is likely that the crude polysaccharide plays role in the gastroprotective activity. The gastroprotective activity of *P. minor* suggests its potential to be developed as a nutraceutical. Further studies to examine other pharmacological activities as well as toxicity testing to show its safety are needed.

6. ACKNOWLEDGEMENTS

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