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Gastroprotective effects of phenylpropanoids from the rhizomes of *Alpinia galanga* in rats: structural requirements and mode of action

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Abstract

The effects of 1′*S*-1′-acetoxychavicol acetate and related phenylpropanoids isolated from the rhizomes of *Alpinia galanga* on ethanol-induced gastric lesions in rats were examined. Among them, 1′*S*-1′-acetoxychavicol acetate and 1′*S*-1′-acetoxyeugenol acetate markedly inhibited the ethanol-induced gastric mucosal lesions (ED_{50} = 0.61 and ca. 0.90 mg/kg). In addition, 1′*S*-1′-acetoxychavicol acetate inhibited the lesions induced by 0.6 M HCl (ED_{50} = 0.73 mg/kg) and aspirin (ED_{50} = 0.69 mg/kg) but it did not show a significant effect on indomethacin-induced gastric lesions and acid output in pylorus-ligated rats at doses of 0.5–5.0 mg/kg. From the gastroprotective effects of various related compounds, the 1′-acetoxyl group of 1′*S*-1′-acetoxychavicol acetate and 1′*S*-1′-acetoxyeugenol acetate was found to be essential for their strong activity. With regard to the mode of action, the gastroprotective effects of 1′*S*-1′-acetoxychavicol acetate were attenuated by pretreatment with indomethacin and *N*-ethylmaleimide, and 1′*S*-1′-acetoxychavicol acetate significantly increased the glutathione levels of gastric mucosa in rats. These findings suggest that endogenous prostaglandins and sulfhydryl compounds are involved in the protective effect of 1′*S*-1′-acetoxychavicol acetate.

Keywords: *Alpinia galanga*; 1′*S*-1′-Acetoxychavicol acetate; Necrotizing agent-induced gastric lesion; Prostaglandin; Sulfhydryl compound

1. Introduction

The Zingiberaceae plant, *Alpinia galanga* SWARTZ, is widely cultivated in China, India, and Southeast Asian countries such as Thailand, Indonesia, and Philippines. The rhizomes of this plant are extensively used as spice or ginger substitutes for flavoring foods, and also used in traditional medicine for several purposes, such as stomachic in China, or for carminative, antiflatulent, antifungal, and anti-itching in Thailand. In chemical studies of *A. galanga*, the pungent principal compound, 1′*S*-1′-acetoxychavicol acetate (**1**), was reported to possess various biological activities, such as antitumor (Itokawa et al., 1987; Kondo et al., 1993; Moffatt et al., 2000; Zheng et al., 2002), antiinflammatory (Nakamura et al., 1998), pungency (Yang and Eilerman, 1999), antifungal (Janssen and Scheffer, 1985), antioxidative (Kubota et al., 2001), and xanthine oxidase inhibitory activity (Noro et al., 1988).

In the course of our studies on the gastroprotective effects of natural medicines (Matsuda et al., 1998, 1999a,b, 2002; Pongpiriyadacha et al., 2003), we found that the 80% aqueous acetone extract from the dried rhizomes of *A. galanga* collected in Thailand showed potent protective effects against ethanol-induced gastric lesions, which was found to be stronger than clinically used medicines, omeprazole, cimetidine, and cetraxate hydrochloride. By bioassay-guided separation, the principal constituents, 1′*S*-1′-acetoxychavicol acetate (**1**) and some related phenylpropanoids (**2**–**7**) were isolated. 1′*S*-1′-Acetoxychavicol acetate (**1**) from the seed of *A. galanga* was reported to possess antiulcer properties in Shay rats when it was intraperitoneally applied (Mitsui et al., 1976). However, the gastroprotective effects of this compound and the extract of rhizomes of *A. galanga* in other experimental models have not been reported. Furthermore, the structure–activity relations and the mode of action of this compound for its gastroprotective effect are unclarified.

In the present study, we describe the protective effect of the extract from the rhizomes of *A. galanga* and its con-

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stituents on gastric lesions induced by necrotizing agents such as ethanol, 0.6 M HCl, aspirin, and indomethacin. In addition, the structural requirements for the strong gastroprotective activity and the mode of action of **1** are discussed.

2. Materials and methods

2.1. Chemicals

1′*S*-1′-Acetoxychavicol acetate (**1**) and some related compounds, 1′*S*-1′-acetoxyeugenol acetate (**2**), 1′*S*-1′-hydroxychavicol acetate (**3**), *trans*-*p*-hydroxycinnamaldehyde (**4**), *trans*-*p*-coumaryl alcohol (**5**), *trans*-*p*-hydroxycinnamyl acetate (**6**), and *trans*-p-coumaryl diacetate (**7**) were isolated from the dried drizomes of *A. galanga* SWARTZ, collected in Thailand (Fig 1).

Thus, the dried rhizomes of *A. galanga* (2.1 kg) were extracted with 80% aqueous acetone three times under room temperature. The aqueous acetone extract (6.6% from this natural medicine) was subjected to ordinary-phase silica-gel (SiO_2) [*n*-hexane-ethyl acetate (EtOAc) (10:1 → 5:1) → EtOAc] and reversed-phase silica-gel (ODS) column chromatographies [methanol (MeOH)–H_2O] and finally HPLC [YMC-Pack ODS-5-A, 250 × 20 mm i.d., MeOH–H_2O or acetonitrile (CH_3CN)–H_2O] to give 1′*S*-1′-acetoxychavicol acetate (**1**, 1.10% from the natural medicine), 1′*S*-1′-acetoxyeugenol acetate (**2**, 0.013%), 1′*S*-1′-hydroxychavicol acetate (**3**, 0.048%), *trans*-*p*-hydroxycinnamaldehyde (**4**, 0.028%), *trans*-*p*-coumaryl alcohol (**5**, 0.052%), *trans*-*p*-hydroxycinnamyl acetate (**6**, 0.021%), and *trans*-*p*-coumaryl diacetate (**7**, 0.003%). Compounds **1**–**7** were identified by comparison of their physical data including ^{1}H- and ^{13}C-NMR spectra with the reported values (Barik et al., 1987; Daubresse et al., 1994; Lee and Ando, 2001; Loubinoux et al., 1989; Noro et al., 1988).

Eugenol acetate (**8**) was obtained by acetylation of **9** with acetic anhydride/pyridine, and demethyleugenol (= hydroxychavicol, **10**) was isolated from *Piper betle* (Lee-Chen et al., 1996). Eugenol (**9**), *trans*-cinnamic acid (**12**), *trans*-*o*-coumaric acid (**13**), and *trans*-*p*-coumaric acid (**15**) were purchased from Nacalai Tesque, Kyoto, Japan. *Trans*-*m*-coumaric acid (**14**) was purchased from Tokyo Kasei Kogyo, Tokyo, Japan. Cinnamaldehyde (**11**), carboxymethyl cellulose sodium salt (CMC-Na), acacia, cimetidine, indomethacin, aspirin, *N*-ethylmaleimide (NEM), N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), glutathione (reduced form, GSH), and the other chemicals were purchased from Wako Pure Chemical, Osaka, Japan.

2.2. Animals

Male Sprague–Dawley rats weighing about 230–250 g were purchased from Kiwa Laboratory Animal, Wakayama, Japan. The animals were housed at a constant temperature of 23±2 °C and were fed a standard laboratory chow (MF, Oriental Yeast, Tokyo, Japan). The animals were fasted for 24–26 h prior to the beginning of the experiment but were allowed free access to tap water. All of experiments were performed with conscious rats unless otherwise noted. The experimental protocols were approved by Experimental Ani-

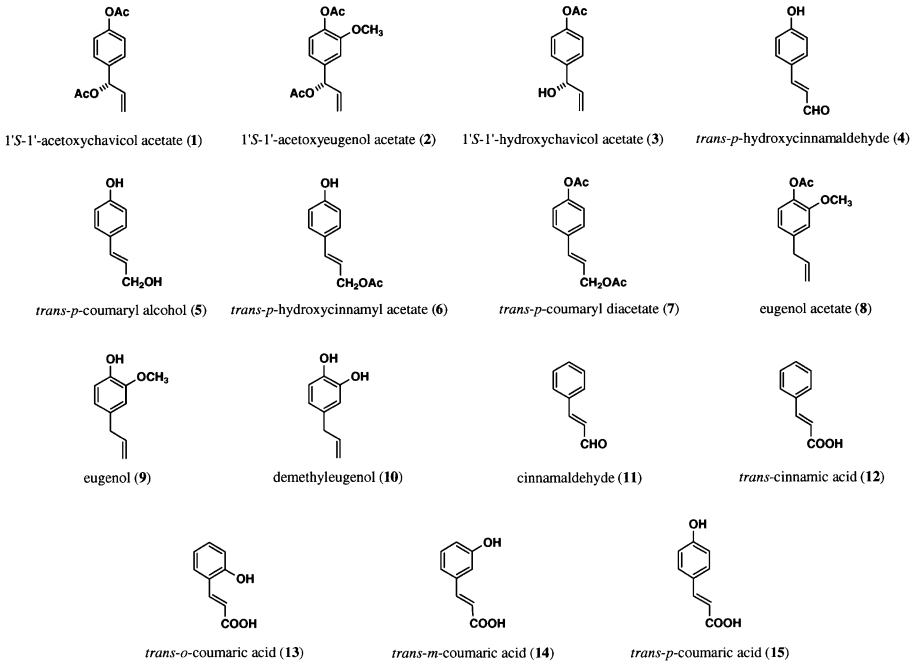

Fig. 1. Chemical structures of phenylpropanoids (**1**–**7**) isolated from *A. galanga* and related compounds (**8**–**15**).

mal Research Committee at Kyoto Pharmaceutical University.

2.3. Ethanol-, 0.6 M HCl-, aspirin-, and indomethacin-induced gastric mucosal lesions in rats

The acute gastric lesions were induced by oral administration of ethanol, 0.6 M HCl, aspirin, and indomethacin according to the method described previously (Morise et al., 1998; Okabe et al., 1974; Robert et al., 1979; Wallace et al., 1990) with slight modifications. Briefly, 99.5% ethanol (1.5 ml/rat), 0.6 M HCl (1.5 ml/rat), aspirin (150 mg/kg suspended in 5% acacia solution and adjusted to 1.5 ml/rat), and indomethacin (20 mg/kg, dissolved in 5% sodium bicarbonate, and then diluted in water and neutralized with 0.2 M HCl and adjusted to 1.5 ml/rat) were administered to 24–26 h fasted rats using a metal orogastric tube. One hour after administration of ethanol and 0.6 M HCl or 4 h after administration of aspirin and indomethacin, the animals were killed by cervical dislocation under ether anesthesia and the stomach was removed and inflated by injection of 10 ml 1.5% formalin to fix the inner and outer layers of the gastric walls. Subsequently, the stomach was incised along the greater curvature and the lengths of gastric lesions were measured as previously described, and the total length (mm) was expressed as a lesion index (Matsuda et al., 1998, 2002; Pongpiriyadacha et al., 2003).

The extract of *A. galanga*, compounds _Hlt40087259 [**1**→**1**, and cetraxate hydrochloride were suspended in 5% acacia solution. Cimetidine and omeprazole were suspended in 0.5% CMC-Na. Test samples in vehicle and vehicle only (control group) were administered orally at a dose of 5 ml/kg 1 h prior to the application of ethanol, 0.6 M HCl, aspirin, and indomethacin.

2.4. Gastric secretion in pylorus-ligated rats

The effect of 1′*S*-1′-acetoxychavicol acetate (**1**) on the gastric acid secretion was examined using pylorus-ligated

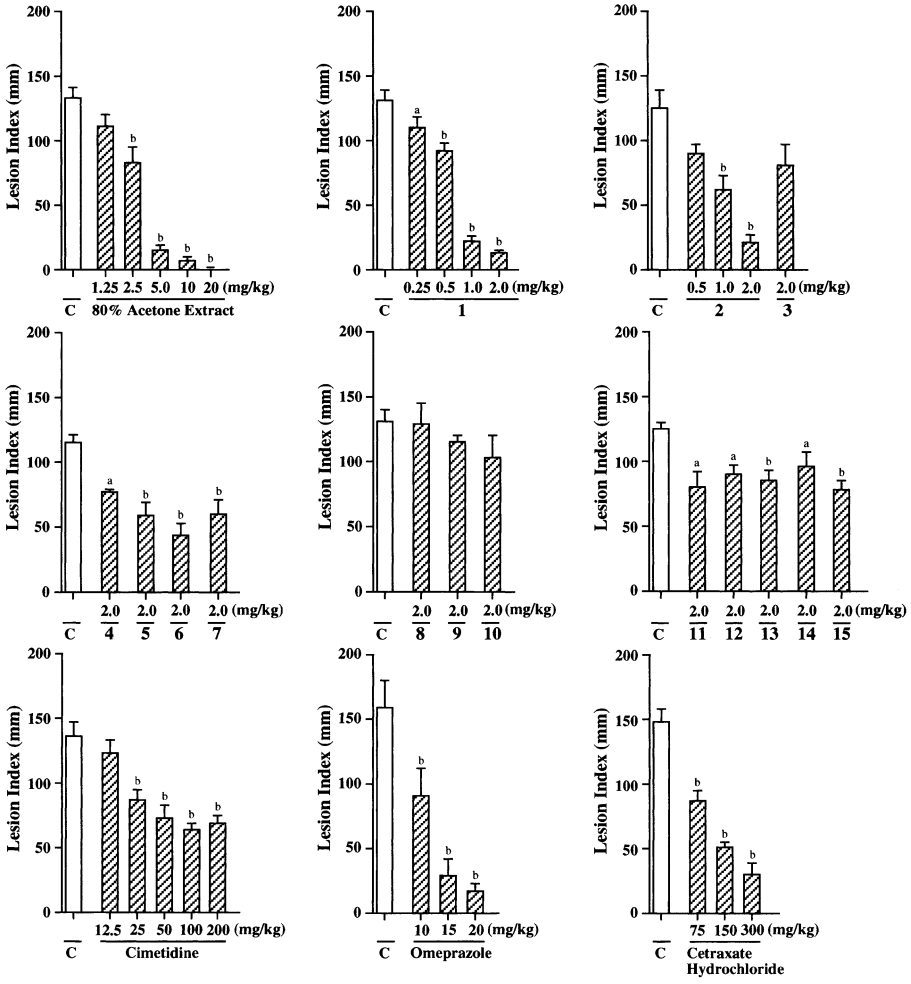

Fig. 2. Effects of 80% aqueous acetone extract and phenylpropanoids (**1**–**7**) from *A. galanga* and related compounds (**8**–**15**) on gastric lesions induced by ethanol in rats. C indicates the control group. The lesion indexes were determined 1 h after administration of ethanol. Each test compound was given orally 1 h before administration of ethanol. Bars represent the means with S.E.M. ($n = 5–8$). Significantly different from the control group, $^{a}P < 0.05$, $^{b}P < 0.01$.

rats. The pyloric ligation was carried out according to the method of Shay et al. (1945). Rats weighing about 240 g were anesthetized with ether, then the abdomen was incised and the pylorus was ligated. Four hours later, the rats were killed and the cardia was ligated. The stomach was removed and the gastric juice was collected in a graduated centrifuged tube. After centrifugation, the volume and pH of the gastric juice were measured. Total acidity was determined by titration with 0.1 M NaOH and acid output (μeq/h) was calculated. Test compounds in vehicle and vehicle only (control group) were administered orally at a dose of 5 ml/kg 1 h prior to the pyloric ligation.

2.5. Ethanol-, 0.6 M HCl-, and aspirin-induced gastric lesions in indomethacin-pretreated rats

The gastric mucosal lesions were induced and the length of gastric lesions were measured as described above. To investigate the involvement of endogenous prostaglandins (PGs) in the protective effect of 1′*S*-1′-acetoxychavicol acetate (**1**), indomethacin (10 mg/kg, dissolved in 5% $NaHCO_3$, and diluted in distilled water, s.c.) was injected 30 min before administration of **1** and vehicle.

2.6. Ethanol-induced gastric lesions in L-NAME- or NEM-pretreated rats

The gastric mucosal lesions were induced, and the lengths of gastric lesions were measured as above method. To investigate the possible involvement of endogenous nitric oxide (NO) and sulfhydryl compounds (SHs) in the protective effect of 1'*S*-1'-acetoxychavicol acetate (**1**), L-NAME, an inhibitor of NO synthase (NOS), (70 mg/kg, dissolved in saline, i.p.) and NEM, an SH-blocker, (10 mg/kg, dissolved in saline, s.c.) were injected 30 min before the administration of 1′*S*-1′-acetoxychavicol acetate (**1**) (Murakami et al., 1996; Szabo et al., 1981).

2.7. Assay of GSH in gastric mucosa

The amount of GSH (nonprotein SHs) in the gastric mucosa was measured according to the method described by Sedlak and Lindsay (1968) and Takeuchi et al. (1989) with slight modifications. Briefly, 1′*S*-1′-acetoxychavicol acetate (**1**) or vehicle was administered to rats 1 h before administration of water or ethanol in each group. The rats were killed 1 h later. The stomachs were incised along the greater curvature and washed gently with cold saline. The

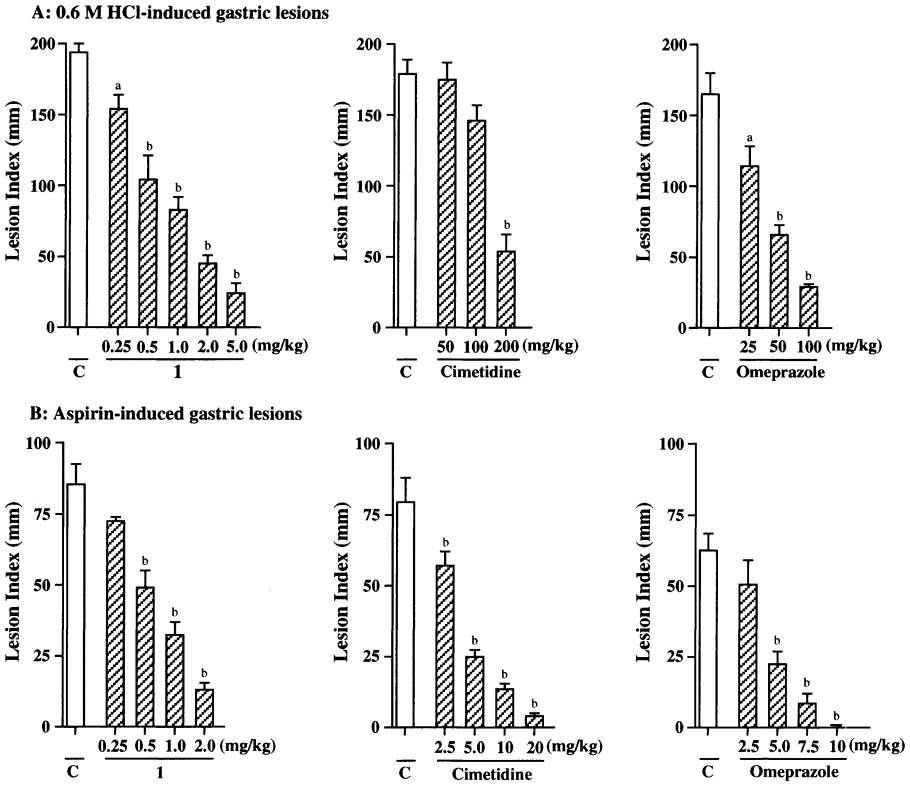

Fig. 3. Effects of 1′*S*-1′-acetoxychavicol acetate (**1**) on gastric lesions induced by 0.6 M HCl (A) and aspirin (B) in rats. C indicates the control group. The lesion indexes were determined 1 and 4 h after administration of 0.6 M HCl and aspirin, respectively. Each test compound was given orally 1 h before administration of 0.6 M HCl and aspirin. Bars represent the means with S.E.M. ($n = 5$ or 6). Significantly different from the control group, $^{a}P < 0.05$, $^{b}P < 0.01$.

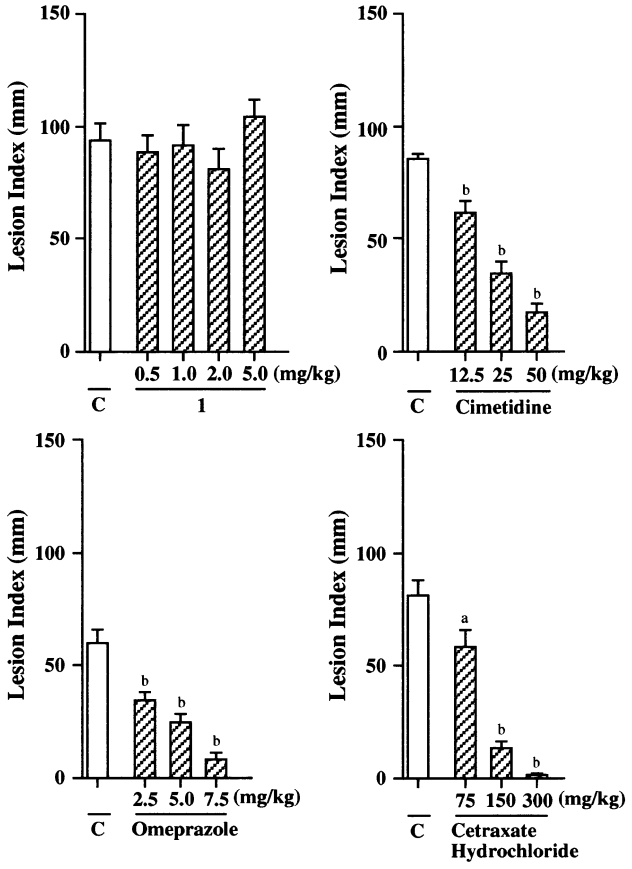

Fig. 4. Effects of 1′*S*-1′-acetoxychavicol acetate (**1**) on gastric lesions induced by indomethacin in rats. C indicates the control group. The lesion indexes were determined 4 h after administration of indomethacin. Each test compound was given orally 1 h before administration of indomethacin. Bars represent the means with S.E.M. ($n=5$ or 6). Significantly different from the control group, $^{a}P<0.05$, $^{b}P<0.01$.

mucosal surface of the stomach was collected by scraping, weighed, and homogenized in 2 ml of 50 mM Tris–HCl buffer containing 20 mM EDTA and 0.2 M sucrose, pH 7.5. The homogenate was centrifuged at 4000 rpm for 30 min at 4 °C, and 0.5 ml of 25% trichloroacetic acid was added to 1 ml of supernatant in each sample and kept for 30 min at 4 °C. After centrifugation at 4200 rpm for 40 min at 4 °C, the supernatant was used to determine GSH using DTNB. Absorbance was measured at 412 nm using a spectrophotometer (Beckmann DU 530). The results of the GSH level in the gastric mucosa were expressed as micromoles per gram wet tissue weight (μmol/g wet weight).

2.8. Statistics

Values were expressed as means ± S.E.M. For statistical analysis, one-way analysis of variance followed by Dunnett's test was used. Probability (P) values less than 0.05 were considered significant. ED_{50} values with 95% confidence limits (95% CL) were estimated based on linear regressions of probit-transformed values of inhibition (%).

3. Results

3.1. Effects of 80% aqueous acetone extract on ethanol-induced gastric lesions

Oral administration of ethanol in a volume of 1.5 milliliter per rat (p.o.) produced gastric mucosal lesions in rats. As shown in Fig. 2, the 80% acetone extract (2.5–20 mg/kg, p.o.) from the rhizomes of *A. galanga* showed strong gastroprotective effect in a dose-dependent manner and almost completely inhibited gastric lesion against ethanol at dose of 20 mg/kg. The ED_{50} (95% CL) of 80% acetone extract was 2.6 (0.80–4.8) mg/kg.

3.2. Effects of 1′S-1′-acetoxychavicol acetate (1) and related compounds on gastric lesions induced by ethanol in rats

1′*S*-1′-Acetoxychavicol acetate (**1**) showed the most gastroprotective effect with ED_{50} (95% CL) of 0.61 (0.17–1.32) mg/kg. Compound **2** also showed potent protective effect on ethanol-induced gastric lesions but its effects tended to be weaker than that of 1′*S*-1′-acetoxychavicol acetate (**1**) (ED_{50} = ca. 0.90 mg/kg). Furthermore, *trans*-*p*-hydroxycinnamaldehyde (**4**), *trans*-*p*-coumaryl alcohol (**5**), *trans*-*p*-hydroxycinnamyl acetate (**6**), and *trans*-*p*-coumaryl diacetate (**7**) also significantly inhibited gastric lesions at a dose of 2.0 mg/kg, whereas

Table 1
Effects of 1′*S*-1′-acetoxychavicol acetate (**1**) from *A. galanga* on gastric secretion in pylorus-ligated rats

Treatment	Dose (mg/kg, p.o.)	*n*	Volume (ml/4 h)	pH	Acid output (μeq/h)
Control	–	9	5.4 ± 0.4	1.56 ± 0.02	133.4 ± 15.5
1′*S*-1′-	0.5	6	3.8 ± 0.4	1.66 ± 0.05	90.3 ± 12.4
Acetoxychavicol	1.0	6	4.7 ± 0.5	1.62 ± 0.03	113.7 ± 21.5
acetate (**1**)	2.0	6	5.3 ± 0.7	1.57 ± 0.05	115.5 ± 22.3
	5.0	6	6.6 ± 0.5	1.59 ± 0.04	117.5 ± 9.2
Control cimetidine	–	9	5.0 ± 0.3	1.49 ± 0.02	133.8 ± 11.2
	50	6	3.8 ± 0.2[a]	1.67 ± 0.05	74.3 ± 6.8[b]
	100	6	3.3 ± 0.4[b]	2.03 ± 0.16[b]	56.6 ± 9.1[b]
Control omeprazole	–	6	4.7 ± 0.4	1.42 ± 0.02	120.3 ± 19.7
	5.0	6	3.3 ± 0.4	1.63 ± 0.03[a]	62.6 ± 10.5[a]
	10	6	2.5 ± 0.4[b]	1.80 ± 0.04[b]	46.8 ± 6.6[b]
	20	6	2.2 ± 0.2[b]	5.54 ± 0.26[b]	14.6 ± 3.6[b]

The pylorus was ligated under ether anesthesia. Four hours later, the animals were killed, and the gastric juice was collected and centrifuged. The volume and pH of the gastric juice were measured. Total acidity was titrated with 0.1 M NaOH and acid output (μeq/h) was calculated. Values represent the means ± S.E.M. Significantly different from the control group, $^{a}P<0.05$, $^{b}P<0.01$.

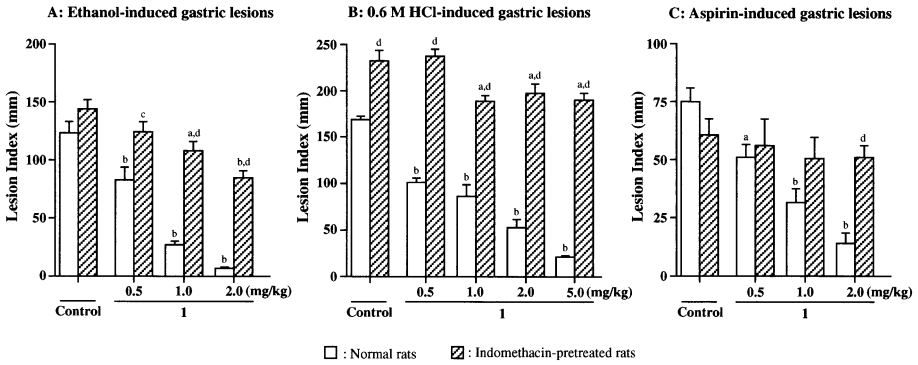

Fig. 5. Effects of 1′*S*-1′-acetoxychavicol acetate (**1**) on gastric lesions induced by ethanol (A), 0.6 M HCl (B), and aspirin (C) in indomethacin-pretreated rats. Indomethacin was administered 30 min before administration of test samples. Ethanol and 0.6 M HCl were administered 1 h and aspirin was administered 4 h after administration of test samples. Bars represent the means with S.E.M. ($n=5-7$). Significantly different from the control group, $^{a}P<0.05$, $^{b}P<0.01$, and from the corresponding group in normal rats, $^{c}P<0.05$, $^{d}P<0.01$.

1′*S*-1′-hydroxychavicol acetate (**3**) and related compounds **8**–**10** lacked the effect, and compounds **11**–**15** showed weak effects at a dose of 2.0 mg/kg. The reference compounds, omeprazole and cetraxate hydrochloride, inhibited the gastric mucosal damage induced by ethanol in a dose-dependent manner. Cimetidine (25–200 mg/kg, p.o.) also showed significant inhibition against ethanol-induced gastric lesions but its maximum inhibition was ca. 50% at doses of 50–200 mg/kg (Fig. 2).

3.3. Effects of 1′S-1′-acetoxychavicol acetate (1) on 0.6 M HCl-, aspirin-, and indomethacin-induced gastric lesions

Oral administration of 0.6 M HCl, aspirin (150 mg/kg), or indomethacin (20 mg/kg) in a volume of 1.5 ml/rat (p.o.) also produced gastric mucosal lesions in rats. 1′*S*-1′-Acetoxychavicol acetate (**1**) (0.25–5.0 mg/kg, p.o.) dose-dependently inhibited the formation of gastric lesions induced by 0.6 M HCl and aspirin but did not show any effect on the lesions induced by indomethacin (Figs. 3 and 4). The ED_{50} (95% CL) of **1** for 0.6 M HCl and aspirin-induced gastric lesions were 0.73 (0.51–0.99) and 0.69 (0.54–0.87) mg/kg, respectively. The reference compounds, cimetidine and omeprazole, inhibited the gastric mucosal damage induced by 0.6 M HCl, aspirin, and indomethacin in a dose-dependent manner.

3.4. Effect of 1′S-1′-acetoxychavicol acetate (1) on gastric secretion in pylorus-ligated rats

The effect of 1′*S*-1′-acetoxychavicol acetate (**1**) on gastric secretion in pylorus-ligated rats is shown in Table 1, compound **1** (0.5–5.0 mg/kg, p.o.) did not inhibit acid output. On the other hand, reference compounds, cimetidine (50 and 100 mg/kg, p.o.) and omeprazole (5–20 mg/kg, p.o.), significantly reduced the acid output while it markedly increased the pH of gastric juice.

3.5. Effects of 1′S-1′-acetoxychavicol acetate (1) on ethanol-, 0.6 M HCl-, or aspirin-induced gastric lesions in indomethacin-pretreated rats

As shown in Fig. 5, pretreatement with indomethacin (10 mg/kg, s.c.) significantly reduced the protective effect of 1′*S*-1′-acetoxychavicol acetate (**1**) on the lesions induced by ethanol, 0.6 M HCl, and aspirin. Practically, in 0.6 M HCl- and aspirin-induced gastric lesions, the protective effects of **1** were almost completely inhibited after pretreatment with indomethacin.

3.6. Effects of 1′S-1′-acetoxychavicol acetate (1) on gastric lesions in L-NAME-pretreated rats

As shown in Fig. 6, pretreatement with L-NAME (70 mg/kg, i.p.) did not reduce the protective effects of 1′*S*-1′-

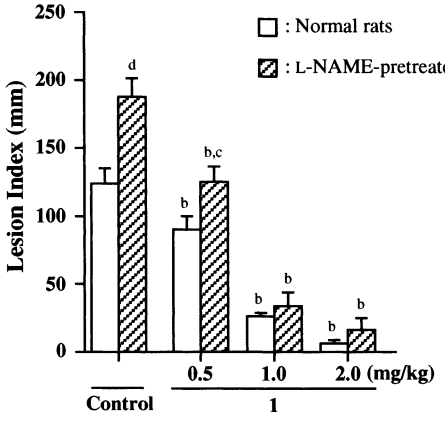

Fig. 6. Effects of 1′*S*-1′-acetoxychavicol acetate (**1**) on gastric lesions induced by ethanol in L-NAME-pretreated rats. L-NAME was administered 30 min before administration of test samples. Ethanol was administered 1 h after administration of test samples. Bars represent the means with S.E.M. ($n=6$). Significantly different from the control group, $^{a}P<0.05$, $^{b}P<0.01$, and from the corresponding group in normal rats, $^{c}P<0.05$, $^{d}P<0.01$.

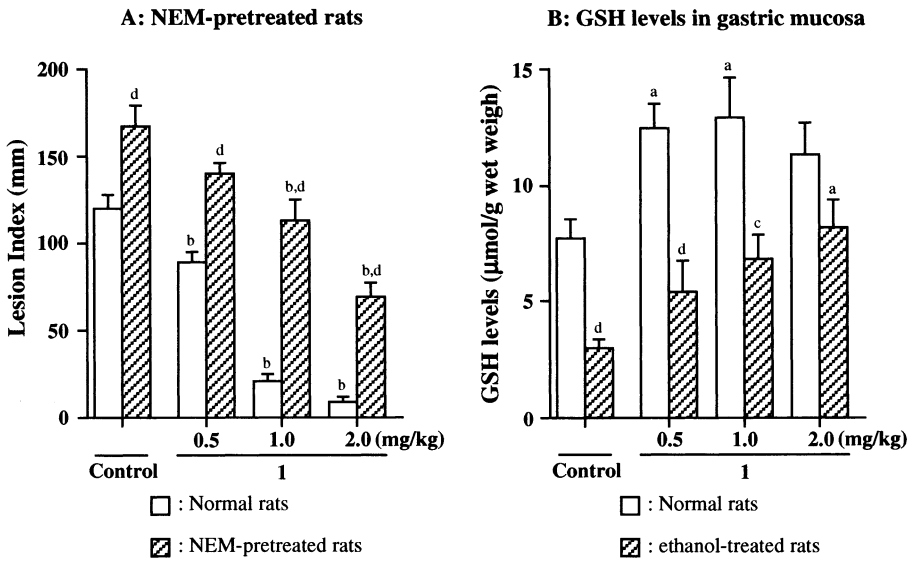

Fig. 7. Effects of 1′*S*-1′-acetoxychavicol acetate (**1**) on ethanol-induced gastric lesions in NEM-pretreated rats (A) and GSH levels in the gastric mucosa (B). (A) NEM was administered 30 min before administration of test samples. Ethanol was administered 1 h after administration of test samples. (B) GSH levels was determined 1 h after administration of water (normal group) and ethanol (ethanol-treated group). Test sample was administered 1 h before administration of water or ethanol. Bars represent the means with S.E.M. ($n=6-9$). Significantly different from the control group, $^{a}P<0.05$, $^{b}P<0.01$, and from the corresponding group in normal rats, $^{c}P<0.05$, $^{d}P<0.01$.

acetoxychavicol acetate (**1**) compared with the inhibition of normal groups at all dose tested.

3.7. Effects of 1′S-1′-acetoxychavicol acetate (1) on gastric lesions induced by ethanol in NEM-pretreated rats and on GSH levels in the rats gastric mucosa after treatment of ethanol

Pretreatment with NEM (10 mg/kg, s.c.) significantly reduced the gastroprotective effect of 1′*S*-1′-acetoxychavicol acetate (**1**) at all doses tested. At the doses of 1.0 and 2.0 mg/kg (p.o.), the protective activities were reduced by about 60% and 36%, respectively (Fig. 7A).

The effects of 1′*S*-1′-acetoxychavicol acetate (**1**) on the amount of GSH level in the gastric mucosa are shown in Fig. 7B. In normal rats, (vehicle + water), compound **1** at doses of 0.5 and 1.0 mg/kg showed significant increase in the GSH level. The mucosal GSH level in the ethanol-treated group was decreased by ca. 60% (from 7.69 ± 0.83 to 2.99 ± 0.36 μmol/g wet weight), whereas compound **1** (2.0 mg/kg) significantly increased the GSH level in gastric mucosa about 3-fold (from 2.99 ± 0.36 to 8.15 ± 1.21 μmol/g wet weight).

4. Discussions

The aqueous acetone extract of the rhizomes of *A. galanga* markedly inhibited the gastric lesions induced by ethanol in rats, and its effect was stronger than those of several synthetic antiulcer drugs, cimetidine, omeprazole, and cetraxate hydrochloride. Among the phenylpropanoids isolated from this herb, 1′*S*-1′-acetoxychavicol acetate (**1**) and 1′*S*-1′-acetoxyeugenol acetate (**2**) strongly inhibited the gastric lesions. *Trans-p*-hydroxycinnamaldehyde (**4**), *trans-p*-coumaryl alcohol (**5**), *trans-p*-hydroxycinnamyl acetate (**6**), and *trans-p*-coumaryl diacetate (**7**) also significantly inhibited gastric lesions at a dose of 2.0 mg/kg. However, 1′*S*-1′-hydroxychavicol acetate (**3**) and the related compounds, eugenol acetate (**8**), eugenol (**9**), and demethyleugenol (**10**) lacked the effects at a dose of 2.0 mg/kg. These results suggested that the 1′-acetoxyl group was essential for the strong inhibition by **1** and **2**.

Compound **1** (0.25–2.0 mg/kg, p.o.) significantly inhibited the formation of gastric lesions induced by 0.6 M HCl and aspirin in addition to ethanol. The ED_{50} values of **1** for ethanol-, 0.6 M HCl-, and aspirin-induced gastric lesions were 0.61, 0.73, and 0.69 mg/kg, respectively. However, for the indomethacin-treated group, it did not show any effect.

An H_2-blocker, cimetidine, and a proton pump inhibitor, omeprazole, which were reported to have not only antisecretion of acid but also cytoprotective effects (Okabe et al., 1986, 1994; Chandranath et al., 2002), inhibited the gastric mucosal damage produced by ethanol, 0.6 M HCl, aspirin, and indomethacin in a dose-dependent manner. Previous study demonstrated that intraperitoneal administration of 1′*S*-1′-acetoxychavicol acetate (**1**) at 2.0 mg/kg reduced the acid output in pyrolus-ligated rats (Mitsui et al. 1976). However, in the present study, oral administration of **1** at 0.5–5.0 mg/kg 1 h before pyloric ligation did not show a significant change in the acid output. These findings suggest that the gastroprotective effects of **1** are acid-independent.

PGs have been shown to have a wide spectrum of gastroprotective activity (Konturek, 1997). Chaudhury and

Robert (1980) reported that mild irritants protected the gastric mucosal damage by releasing endogenous PGs. Pretreatment with indomethacin markedly attenuated the gastroprotective effect of 1′*S*-1′-acetoxychavicol acetate (**1**) on the gastric lesions induced by ethanol, 0.6 M HCl, and aspirin. These results suggest that PGs are involved in the gastroprotection by **1**.

NO is known as a potent vasorelaxing factor secreted from endothelial cells or the sensory nerve ending in the mucosa (Peskar et al., 1991). NO participated in the gastric defense mechanism by regulating the gastric mucosal blood flow (Pique et al., 1989), gastric mucus secretion (Brown et al., 1993), and increase in PGs biosynthesis (Uno et al., 1997). In the present study, treatment with L-NAME did not attenuate the protective effect of **1**. This finding suggests that NO is not involved in the protective effect of **1**.

Oxygen-derived free radicals and lipid peroxidation are associated with gastrointestinal lesions, and antioxidants prevent the lesions by various ulcerogens. Gastric mucosal SHs including GSH act as antioxidants, and are important for maintenance of mucosal integrity in the stomach (López et al., 1996; Szabo et al., 1992). Ethanol-induced gastric damage is also associated with a significant decrease in the mucosal SHs level such as GSH, and pretreatment with SH-blockers prevents the gastroprotection of SH-containing compounds (Szabo and Brown, 1987; Szabo et al., 1981). In agreement with previous findings, the concentration of GSH in the gastric mucosa significantly decreased after administration of ethanol, and compound **1** inhibited the decrease in the GSH levels. In addition, pretreatment with an SH-blocker, NEM, significantly reduced the mucosal protection afforded by **1** at all doses tested. These findings suggest that the increase in endogenous SHs is involved in the protective effect of **1**.

In conclusion, 1′*S*-1′-acetoxychavicol acetate (**1**) and 1′*S*-1′-acetoxyeugenol acetate (**2**) from the rhizomes of *A. galanga* markedly inhibited the gastric mucosal lesions induced by ethanol. In addition, compound **1** also inhibited the gastric lesions induced by 0.6 M HCl and aspirin but did not show any effect on indomethacin-induced gastric lesions and acid output in pylorus-ligated rats at doses of 0.5–5.0 mg/kg. From the gastroprotective effects of various related compounds, the 1′-acetoxyl group of **1** and **2** was found to be essential for their strong activity. With regard to the mode of action, the gastroprotection of **1** was attenuated by pretreatment with indomethacin and NEM. Compound **1** significantly increased the GSH levels of gastric mucosa in rats. These findings suggest that endogenous PGs and SHs are involved in the protective effect of **1**. The detail mechanisms of action of **1** need to be studied further.

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