RESULTS

The anti-ulcer activity of the compound: labda-7,12(E),14-triene-17-oic acid (DS4) was investigated in rats using various experimental models, which included 1) EtOH/HCl-, 2) indomethacin-, and 3) restraint water immersion stress-induced gastric lesions. DS4 at the doses of 25, 50 and 100 mg/kg were tested, and cimetidine (H_2-antagonist, 50 and 100 mg/kg) was used as a reference drug.

EtOH/HCl-induced gastric lesions in rats

Administering of EtOH/HCl clearly produced necrosis of mucus membrane lesions in the glandular portion of the stomach as evidenced in the control group. The lesions were found in the mucosa and consisted of elongated bands. The rats which received pretreatment with DS4 or cimetidine had less gastric lesions than those of the control group.

The presence of ulcers, expressed as an ulcer index, of groups pretreated with DS4 and cimetidine (administered intraperitoneally) is shown in Table 1a. Induction of ulcers by EtOH/HCl as observed in the control group resulted in an ulcer index of 172 mm. The ulcer index of DS4 at the doses of 25, 50 and 100 mg/kg were 147, 104 and 45 mm, respectively. Statistically significant decreases of the ulcer index were found only with the doses of 50 and 100 mg/kg. Percent inhibition of ulcer formation increased with the increasing dose of DS4. The percent inhibitions of 14, 39 and 73 were found with the doses of 25, 50 and 100 mg/kg, respectively. Cimetidine showed an anti-ulcer activity with the ulcer index of 107 mm, and percent inhibition of 37.

The effects of DS4 as well as cimetidine administered by an oral route at the dose of 100 mg/kg were assessed on the EtOH/HCl induced gastric ulceration model. As seen in Table 2a, the ulcer index of the control group was 169 mm, whereas that of

the DS4 treated group was 52 mm with percent inhibition of 69. The group receiving cimetidine had an ulcer index of 41 mm, and percent inhibition of 75.

Restraint water immersion stress-induced gastric lesions in rats

When vehicle-treated rats were subjected to 5 h hypothermic stress, marked gastric mucosal damage confined to the glandular segment of the stomach was seen. The lesions consisted of round or elongated well-defined hemorrhagic erosions with petechiae spread throughout the glandular region. Pretreatment with DS4 and cimetidine caused a reduction of restraint water immersion stress-induced gastric lesions.

The ulcer index of DS4, administered intraperitoneally at the doses of 25, 50 and 100 mg/kg, were 11, 8 and 1 mm, respectively, whereas that of the control group was 23 mm (Table 2a). The decreases of the ulcer index were statistically significant. The percent inhibitions of 50, 65 and 94 were found with the doses of 25, 50 and 100 mg/kg. Cimetidine exhibited an anti-ulcer activity, the index decreased to 10 mm, with percent inhibition of 55.

Additionally, the effect of an oral administration of DS4 or cimetidine on gastic ulcerations was evaluated in the restraint water immersion stress model. Data obtained are illustrated in Table 2b. Induction of ulcers by restraint water immersion stress as observed in the control group resulted in an ulcer index of 26 mm. A statistically significant decrease of the ulcer index was found with DS4 administered at the dose of 100 mg/kg, with the ulcer index of 9 mm, and percent inhibition of 62. Cimetidine showed an anti-ulcer activity with the ulcer index of 7 mm, and percent inhibition of 71.

Table 1 Effect of DS4 (labda-7,12(*E*),14-triene-17-oic acid) on EtOH\HCl–induced gastric lesions in rats

a. Intraperitoneal administration of DS4

Group	Ulcer index (mm)	% inhibition
Control (vehicle)	172.13 ± 13.42	-
Cimetidine		
50 mg/kg	107.67 ± 10.11*	37.45
DS4		
25 mg/kg	147.17 ± 13.25	14.50
50 mg/kg	104.55 ± 12.14*	39.26
100 mg/kg	45.00 ± 11.81*	73.86

Each value represents the mean ± S.E.M. from 6 animals in each group. Cimetidine and DS4 were given intraperitoneally 1 h before an oral administration of EtOH/HCl.
* Significantly different from the control group, $P < 0.05$

Table 1 *(continued)* Effect of DS4 (labda-7,12(*E*),14-triene-17-oic acid) on EtOH\HCl–induced gastric lesions in rats

b. Oral administration of DS4

Group	Ulcer index (mm)	% inhibition
Control (vehicle)	169.24 ± 26.77	-
Cimetidine		
100 mg/kg	41.06 ± 13.13*	75.73
DS4		
100 mg/kg	52.38 ± 14.05*	69.05

Each value represents the mean ± S.E.M. from 5 animals in each group. Cimetidine and DS4 were given orally 1 h before an oral administration of EtOH/HCl. * Significantly different from the control group, $P < 0.05$

Table 2 Effect of DS4 (labda-7,12(*E*),14-triene-17-oic acid) on restraint water immersion stress–induced gastric lesions in rats

a. Intraperitoneal administration of DS4

Group	Ulcer index (mm)	% inhibition
Control (vehicle)	23.57 ± 1.97	-
Cimetidine		
50 mg/kg	10.50 ± 2.46*	55.45
DS4		
25 mg/kg	11.68 ± 3.15*	50.52
50 mg/kg	8.18 ± 2.65*	65.28
100 mg/kg	1.35 ± 0.50*	94.27

Each value represents the mean ± S.E.M. from 6 animals in each group. Cimetidine and DS4 were given intraperitoneally 1 h before performing restraint water immersion.
* Significantly different from the control group, $P < 0.05$

Table 2 *(continued)* Effect of DS4 (labda-7,12(*E*),14-triene-17-oic acid) on restraint water immersion stress–induced gastric lesions in rats

b. Oral administration of DS4

Group	Ulcer index (mm)	% inhibition
Control (vehicle)	26.26 ± 1.89	-
Cimetidine		
100 mg/kg	7.43 ± 1.61*	71.71
DS4		
100 mg/kg	9.73 ± 0.95*	62.95

Each value represents the mean ± S.E.M. from 7 animals in each group. Cimetidine and DS4 were given orally 1 h before performing restraint water immersion.
* Significantly different from the control group, $P < 0.05$

Indomethacin-induced gastric lesions in rats

Intraperitoneal administration of indomethacin (control group) resulted in gastric mucosal damage (ulceration) occurring mainly in the glandular segment of the stomach, and the majority of the lesions were petechial erosions. Pretreatment with DS4 administered intraperitoneally effectively reduced the intensity of ulceration induced by indomethacin (Table 3). Administering of indomethacin caused gastric ulceration with an ulcer index of 30 mm (control group). Statistically significant decreases of ulcer index were observed with DS4 administered at the doses of 25, 50 and 100 mg/kg with the ulcer index of 12, 10 and 8 mm, respectively. Percent inhibition of ulcer formation increased with the increasing dose of DS4, and the percent inhibitions of 57, 65 and 71 were found with the doses of 25, 50 and 100 mg/kg, respectively. The ulcer index of 42 mm and percent inhibition of 65 were observed with cimetidine administration.

Anti-gastric ulcer activity of DS4 in different experimental models

Figure 10 depicts the anti-gastric ulcer activity of DS4 and cimetidine administered at the dose of 50 mg/kg when tested in 3 experimental models: EtOH/HCl-, indomethacin- and restraint water immersion stress-induced gastric ulcer. In EtOH/HCl-induced gastric ulcer model, percent inhibitions of 39 and 37 % were observed with the administration of DS4 and cimetidine, respectively. Both DS4 and cimetidine showed an anti-ulcer activity of 65 % when tested in indomethacin-induced gastric ulcer model. In the restraint water immersion stress-induced gastric ulcer model, DS4 was more effective than cimetidine, causing 65 % inhibition, whereas cimetidine showed 55% inhibition.

Table 3 Effect of DS4 (labda-7,12(*E*),14-triene-17-oic acid) on indomethacin–induced gastric lesions in rats

Group	Ulcer index (mm)	% inhibition
Control (vehicle)	30.06 ± 6.66	-
Cimetidine		
50 mg/kg	10.42 ± 1.25*	65.34
DS4		
25 mg/kg	12.76 ± 3.26*	57.55
50 mg/kg	10.48 ± 2.92*	65.14
100 mg/kg	8.66 ± 2.68*	71.19

Each value represents the mean ± S.E.M. from 5 animals in each group. Cimetidine and DS4 were given intraperitoneally 1 h before intraperitoneal injection of indomethacin.

* Significantly different from the control group, $P < 0.05$

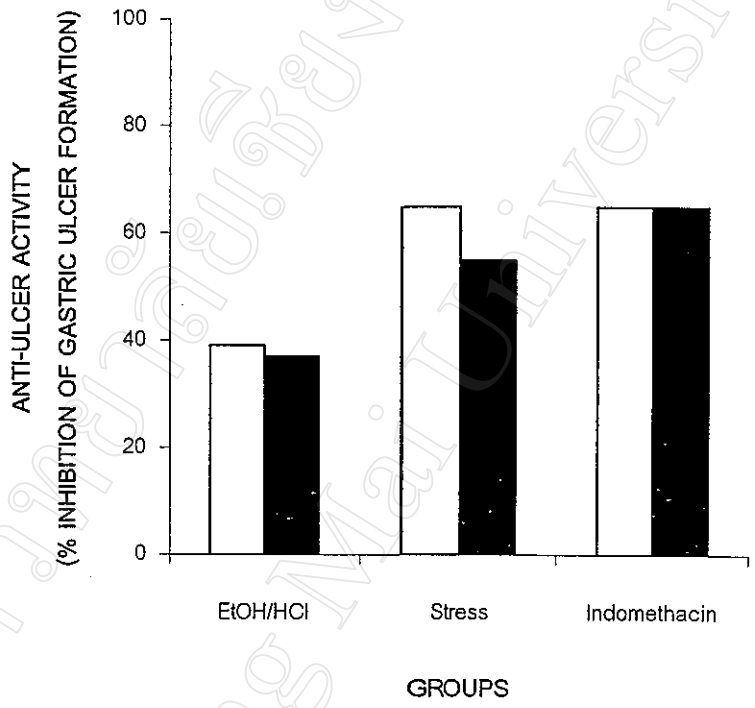

Figure 10 Anti-ulcer activity (% inhibition of gastric ulcer formation) of DS4 (labda-7,12 (*E*),14-triene-17-oic acid) when evaluated in different models. DS4 (□) and cimetidine (■) at the doses of 50 mg/kg were tested in 3 models EtOH/HCl-, restraint water immersion stress- and indomethacin- induced gastric ulcers in rats

Potency of DS4: Comparison between oral and intraperitoneal administration

The potency ratios (ratio of anti-ulcer activity of cimetidine to anti-ulcer activity of DS4) administered orally (at the dose of 50 mg/kg), and intraperitoneally (at the dose of 100 mg/kg) were determined in the EtOH/HCl- and restraint water immersion stress-induced gastric lesions models. The anti-gastric ulcer activity, expressed as percent inhibition of gastric lesion formation, and potency ratios are shown in Table 4.

The oral administration of cimetidine and DS4 resulted in percent inhibitions of 75 and 69, respectively, when the gastric lesions were induced by EtOH/HCl, and the potency ratio was calculated to be 1:0.91. In the case of intraperitoneal administration, cimetidine and DS4 showed showed an anti-ulcer activity with percent inhibitions of 37 and 39, respectively, and the potency ratio was found to be 1:1.05.

In the restraint water immersion stress–induced gastric ulcer model, cimetidine and DS4 administered orally caused percent inhibition of 71 and 62, respectively, and potency ratio was 1:0.88. Intraperitoneal injection of cimetidine and DS4 led to percent inhibitions of 55 and 65, respectively, and a potency ratio of 1:1.18.

Potency ratio values are practically the same, when DS4 was administered intraperitoneally or orally, and when assessed in the EtOH/HCl- and the restraint water immersion stress–induced gastric ulcer models.

Table 4 Comparison of potency of DS4 in the administration of oral and intraperitoneal in EtOH/HCl- and restraint water immersion stress-induced gastric lesions in rats

Route of Administration	Experimental Model			
	EtOH/HCl		Restraint water immersion stress	
	% inhibition of ulcer formation	Potency ratio	% inhibition of ulcer formation	Potency ratio
Oral administration				
Cimetidine 100 mg/kg	75.73	1:0.91	71.71	1:0.88
DS4 100 mg/kg	69.05		62.95	
Intraperitoneal administration				
Cimetidine 50 mg/kg	37.45	1:1.05	55.45	1:1.18
DS4 50 mg/kg	39.26		65.28	

Pylorus ligation experiment

The pylorus ligation initiated ulcer formation with few hemorrhagic spots. However, the ulcer index of the groups receiving DS4 at the doses of 25, 50 and 100 mg/kg did not show any statistically difference from that of the control group.

Table 5 illustrates the effect of DS4 and cimetidine on gastric secretion rate and total acidity. The gastric secretion rate of 0.49 ml/100g/h and total acidity of 7.72 μEq/100g/h were observed in the control group (Vehicle). Pretreatment with DS4 at the doses of 25, 50 and 100 mg/kg significantly decreased gastric secretion rate to 0.11, 0.08 and 0.07 ml/100g/h, respectively. Significant decreases of total acidity of 5.51 and 5.09 μEq/100g/h were observed with DS4 at the doses of 50 and 100 mg/kg, respectively. Total acidity of the group pretreated with DS4 at the dose of 25 mg/kg was 5.94, and was not statistically different from that of the control group. The cimetidine group showed a significant decrease of total acidity (4.06 μEq/100g/h) with a gastric secretion rate of 0.22ml/100g/h, which was not statistically different from that of the control group.

Table 5 Effect of DS4 (labda-7,12(*E*),14-triene-17-oic acid) on gastric acid secretion in pylorus-ligated rats

Group	Ulcer index (mm)	gastric secretion rate (ml/100g/h)	Total acidity (μEq/100g/h)
Control (Vehicle)	2.74 ± 1.15	0.49 ± 0.12	7.72 ± 0.78
Cimetidine			
50 mg/kg	5.30 ± 1.33	0.22 ± 0.10	4.06± 0.44*
DS4			
25 mg/kg	4.45 ± 1.68	0.11 ± 0.06*	5.94 ± 0.68
50 mg/kg	4.42 ± 1.58	0.08 ± 0.04*	5.51± 0.22*
100mg/kg	2.38 ± 1.58	0.07 ± 0.04*	5.09± 0.61*

Each value represents the mean ± S.E.M. from 6 animals in each group. Cimetidine and DS4 were given intraperitoneally 1 h before performing pylorus ligation.
* Significantly different from the control group, $P < 0.05$

Effect of DS4 on gastric wall mucus of EtOH/HCl treated rats

DS4 (25, 50 and 100 mg/kg), cimetidine (50 mg/kg) and prostaglandin (50 and 100 μg/kg) were investigated for their effects on gastric wall mucus of EtOH/HCl treated rats. The data obtained are demonstrated in Table 6.

Gastric wall mucus of the rats which received no EtOH/HCl treatment (normal group) was 137 μg alcian blue/g wet stomach. The treatment with EtOH/HCl resulted in a reduction of gastric wall mucus. As seen in the group receiving vehicle (EtOH/HCl treated group), the mucus (90 μg alcian blue/g wet stomach) was significantly less than that of the normal group (no EtOH/HCl treated group). Pretreatment with DS4 or prostaglandin could protect the loss of gastric wall mucus caused by EtOH/HCl. The gastric wall mucus of the rats receiving DS4 pretreatment at the doses of 25, 50 and 100 mg/kg were found to be 140, 144 and 153 μg alcian blue/g wet stomach, respectively, which were significantly higher than that of control group.

Gastric wall mucus of EtOH/HCl treated group receiving cimetidine was 93 μg alcian blue/g wet stomach and not significantly different from that of the control group. In the groups receiving misoprostol (PGE_1) at the dose of 50 and 100 μg/kg the amounts of the gastric wall mucus were 107 and 148 μg alcian blue/g wet stomach, respectively, and only the amount of mucus of the group receiving 100 μg/kg was statistically higher than that of the control group.

Table 6 Effect of DS4 (labda-7,12(*E*),14-triene-17-oic acid) on gastric wall mucus in rats with EtOH\HCl

Group	Gastric wall mucus (μg alcian blue/g wet stomach)
Normal	137.96 ± 15.31
EtOH/HCl treated rats	
Control (vehicle)	90.85 ± 5.57 [a]
Cimetidine	
50 mg/kg	93.76 ± 7.69
Misoprostol	
50 μg/kg	107.37 ± 6.75
100 μg/kg	148.55 ± 8.97*
DS4	
25 mg/kg	140.46 ± 18.64*
50 mg/kg	144.76 ± 11.75*
100 mg/kg	153.88 ± 20.88*

Each value represents the mean ± S.E.M. from 5 animals in each group. Cimetidine, misoprostol and DS4 were given intraperitoneally 1 h before an oral administration of EtOH/HCl. [a] Significantly different from the normal group, $P < 0.05$, * Significantly different from the control group, $P < 0.05$

Hippocratic screening of DS4

Oral administration of DS4 at the dose of 1 g/kg caused following signs:

1. decreased motor activity, graded as +1 (did not move spontaneously, but when handled will move rapidly).
2. analgesia, graded as +2 (no response when the pin was firmly pressed down across the instep and toes of one of the rat 's hind feet).

After 7 days of observation, the surviving rats were killed and necropsy was performed. Normal color and normal size of internal organs were observed.