DISCUSSION

The peptic ulcer has been postulated to be resulted from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms. Aggressive factors are HCl, gastrin, histamine, *H. pylori*, aspirin and other NSAIDs, ethanol, caffeine and stress. Defensive factors are gastric mucus and bicarbonate, gastric mucosal barrier, PGs, and mucosal blood flow (Brunton, 1996; Friedmand and Peterson, 1998).

In the present study, DS4 (labda-7,12(*E*),14-triene-17-oic acid, a labdane compound isolated from *Croton oblongifolious*) at the doses of 25, 50 and 100 mg/kg showed an anti-ulcer activity when evaluated by various experimental models, which included EtOH/HCl-, restraint water immersion stress- and indomethacin-induced gastric ulcers in rats. Additionally, DS4 caused a decrease of gastric secretion and acidity when tested in a pylorus ligation experiment, and increased gastric wall mucus of rats with EtOH/HCl- induced gastric ulcers.

The EtOH/HCl-, restraint water immersion stress- and indomethacin-induced gastric ulceration in rats are among the most commonly utilized experimental models for evaluation of anti-ulcer activity in rats (Robert *et al.*, 1979; Murakami *et al.*, 1985), and various mechanisms are implied to be associated with the formation of gastric mucosal damage (gastric ulceration) in these experimental models.

In the model EtOH/HCl-induced gastric lesion, HCl caused severe damage to gastric mucosa (Yamahara *et al.*, 1988). Additionally, ethanol produced necrotic lesions in the gastric mucosa by its direct toxic effect causing reductions of defensive factors, bicarbonate secretion and mucus production (Marhuenda *et al.*, 1993). Development of ethanol-induced gastric hemorrhagic lesions has been shown to be preceded by an early vascular damage with stasis of gastric blood flow and increase mucosal

microvascular permeability (Guth *et al.*, 1984; Ohya and Guth, 1988 and Takeuchi *et al.*, 1989). Ethanol is known to stimulate the formation of leukotriene C_4 (LTC_4), a lipoxygenase-derived metabolite of arachidonic acid (Hua *et al.*, 1985) and products of the 5-lipoxygenase pathway possibly play a key role in the development of the ethanol-induced ulceration (Lange *et al.*, 1985). Interestingly leukotriene antagonists and 5-lipoxygenase inhibitors showed an inhibitory effect on ethanol and NSAIDs-induced gastric ulceration in rats (Parnaham and Brune, 1987).

The cytoprotective action of many drugs has been demonstrated in the animal models of acute gastric injury induced by necrotizing agents such as ethanol, HCl and ethanol/HCl, etc. (Konturek *et al.*, 1984). In addition, the cytoprotective action of some anti-ulcer drugs and the action of mild irritants has been suggested to be mediated by the action of endogenous prostaglandins which are known to play an important role in maintaining mucosal integrity (Miller, 1983) and to protect the gastric mucosal against various damaging agents (Robert *et al.*, 1983). Moreover, a protective effect of prostaglandins against the formation of gastric mucosal lesions induced by various necrotizing agents (Robert *et al.*, 1979; Yamamoto *et al.*, 1991) have also been shown.

DS4 at the doses of 25, 50 and 100 mg/kg showed an anti-ulcer activity causing a reduction of ulcer formation induced by EtOH/HCl. It is possible that DS4 possesses a gastric mucosal membrane protective action according to the mechanism stated by Konturex *et al.* (1984) and Lange *et al.* (1985) for drugs which are effective against EtOH/HCl-induced gastric lesions. The protective mechanism is probably mediated by increasing mucosal resistance (by increasing gastric blood flow) or potentiation of defensive factors (i.e mucus). Additionally, it is also probable that the protective action of DS4 against ethanol-induced gastric lesions is mediated by an inhibition of the 5-lipoxygenase pathway or by leukotriene antagonistic activity. However, these assumptions need confirmation.

The model, "restraint water immersion stress-induced gastric ulcers in rats," has been widely used experimentally for the evaluation of anti-ulcer activity because of data

reproducibility (Murakami *et al.*, 1985). Mechanisms such as disturbance of gastric mucosal microcirculation (Guth, 1972), alteration of gastric secretion (Kitakawa *et al.*, 1979) and abnormal gastric motility (Watanabe, 1966) have been proposed to be involved in stress-induced gastric mucosal lesions. Besides, vagal overactivity is suggested to be an effector in stress-induced ulceration because the stress can be prevented partly or entirely by vagotomy (Brodie and Hanson, 1960). Acetylcholine from the vagus nerve and other endogenous substances such as histamine from mast cells, and gastrin from G cells are known to affect HCl secretion by stimulating the parietal cells directly to secrete HCl (Bullock and Boyle, 1995). Furthermore, most of the stressful stimuli increase ACTH secretion from the anterior pituitary via the release of CRH from the hypothalamus and consequently, a rise in the circulating glucocorticoids level. Glucocorticoids can produce peptic ulcers probably by reducing the formation of PGE_2 and PGI_2 leading to a decreased mucus secretion in the stomach (Ganong, 1997).

DS4 significantly inhibited gastric ulcer induced by restraint water immersion stress. The protective action of DS4 against stress induced-ulceration could probably be due to an inhibitory effect on gastric secretion effect through cholinergic receptor and/or increased prostaglandins formation thus leading to increased mucus (defensive factor) secretion.

DS4 exhibited an anti-gastric ulcer activity when assessed in rats with indomethacin-induced gastric ulcer. Indomethacin and other NSAIDs can cause peptic ulcer during the course of their anti-inflammatory action. NSAIDs inhibit cyclooxygenase (COX) leading to reduction of PG synthesis from arachidonic acid consequently leading to over production of leukotrienes and other products of 5-lipoxygenase pathway (Rainsford, 1987). High concentrations of PGs, especially PGE_2 and PGI_2, are present in the normal gastric and duodenal mucosa and they are responsible for mucus production. Inhibition of PG synthesis by traditional NSAIDs such as indomethacin which are non-selective COX inhibitors (inhibit both COX-1 and COX-2), causes gastric and intestinal ulceration and delays gastric ulcer healing in chronic ulcer (Robert *et al.*,

1983). Other mechanisms of indomethacin-induced stomach ulcers have also been well documented. These include a reduction in local blood flow, topical irritation (when given orally) and an interference with restitution and tissue repair (Ito and Lacy, 1985; Hudson *et al.*, 1991; Lau *et al.*, 1992; Lanza *et al.*, 1995; Teha *et al.*, 1995).

Robert *et al.*(1979) coined the term 'cytoprotection' to describe the property of prostaglandins and other compounds which have no structural similarity with PGs by which cells are rendered defensive to stave off gastric mucosal lesions induced by various necrotizing agents such as ethanol, strong acid or base and NSAIDs like indomethacin and aspirin. This pharmacological action is achieved independently of the inhibition of gastric acid secretion.

Since DS4 was found to significantly inhibit gastric ulcer induced by indomethacin, it is likely that anti-ulcer activity is associated with an increase of endogenous PGs thereby increasing gastric mucus. In addition, leukotriene antagonism might contribute to the gastroprotective effect of DS4.

It was noted that DS4 and cimetidine exerted anti-gastric ulcer activity with comparable percent inhibition of ulcer formation when evaluated in experimental models used in the present study. Similarly to cimetidine the activity was lowest when tested in the EtOH/HCl-induced gastric ulcer model.

Most of the anti-gastric ulcer drugs are usually administered by an oral route. In the present study, DS4 was administered intraperitoneally when assessed for anti-gastric ulcer activity in all of the experimental models because of smaller amount of DS4 required. However, DS4 has been found to be orally active since the its anti-gastric ulcer activity could be confirmed when tested in the EtOH/HCl–, and restraint water immersion stress-induced gastric ulcer models (Table 1b & 2b). In addition, the potency of DS4 compared to cimetidine (expressed as potency ratio), administered orally and intraperitoneally was practically the same (Table 4).

DS4 exhibited a protective effect against restraint water immersion stress-induced as well as indomethacin-induced gastric lesions. The anti-gastric ulcer or gastroprotective effect might possibly be due to an increase of gastric mucus (a defensive factor against gastrointestinal damage) and/or a decrease of gastric acid secretion (aggressive factor). Thus, further study was then carried out to explore the effects of DS4 on gastric wall mucus of gastric ulcerated rats and on gastric acid secretion of pylorus ligated rats.

The pylorus ligation in rat experiment is a model used for examining anti-secretory activity (Shay *et al.*, 1945) of anti-gastric ulcer agents. The ligation caused an accumulation of intraluminal HCl, leading to gastric mucosal damage. In addition, the gastric acid output as well as volume of gastric content can also be determined. In the present study, DS4 and cimetidine exhibit an anti-secretory activity causing significant decreasing of both gastric volume and total acidity (Table 5).

The effect of DS4 on gastric wall mucus was studied in EtOH/HCl-induced gastric ulcer model. The mucus content in gastric tissue was estimated by measuring the amount of alcian blue bound to mucus spectrophotometrically at 580 nm (Corn *et al.*, 1974). Cimetidine did not increase gastric wall mucus. A significant increase of gastric wall mucus was observed with DS4 when tested at the doses of 25, 50 and 100 mg/kg and with a PGE_1 analogue: misoprostol (at the dose of 100 ug/kg). Thus, it is likely that DS4 similarly to misoprostol prevents EtOH/HCl - induced gastric damage by increasing mucus production. An increased mucus production mediating cytoprotection is generally known to be a mechanism of anti-gastric ulcer activity of PGE (Robert *et al.*, 1983). It is therefore reasonable to assume that DS4 exerts anti-gastric ulcer activity by cytoprotective action.

The present study has demonstrated several features of the anti-gastric ulcer effect of DS4. According to the results obtained, anti-ulcer activity offered by DS4 is mediated via mechanisms which include a stimulation of mucus secretion (potentiation of defensive factors), an inhibition of gastric acid output (attenuation of aggressive

factors). The gastroprotective (cytoprotective) mechanism of DS4 via an increase in gastric mucus is attributed to its anti-ulcer effect. Additionally, DS4 showed an anti-oxidant activity when tested on $FeCl_2$-ascorbic acid stimulated lipid peroxidation in rat liver homogenate (unpublished data, the study carried out by staff of the Laboratory of Natural Products, Chulabhorn Research Center at Chiang Mai University), thus the anti-oxidant activity might possibly play a role in the anti-ulcer activity of DS4. The studies conducted by Konturek *et al.* (1988) and Dajani and Agrawal, (1995) have pointed out the roles of leukotrienes, lipoxygenase-derived metabolites of arachidonic acid, in the experimental models of gastric lesions induced by ethanol, indomethacin and stress. Thus, it is not possible to altogether exclude that the anti-ulcer effect of DS4 is mediated by an inhibition of a 5-lipoxygenase pathway or leukotriene antagonistic activity. However, these assumptions need confirmation.

In addition to anti-gastric ulcer activity, DS4 was subjected to the Hippocratic screening test. The test is commonly used as a preliminary screening to detect interesting pharmacological activity and also signs of toxicity (Malone, 1977). DS4 shows anti-gastric ulcer activity when tested at the dose of 100 mg/kg administered orally. According to the results obtained, it is likely that DS4 exhibits low toxicity. An oral administration of DS4 at the dose 1000 mg/kg did not cause death, but was found to cause a decrease of motor activity and analgesia, thus suggesting a central nervous system (CNS) depressant activity.

Further studies are warranted to investigate the toxicity and detailed mechanisms of action of this substance before being submitted for clinical trial study.