INTRODUCTION

Peptic ulcers are chronic, most often solitary, lesions that occur in any portion of the gastrointestinal tract exposed to the aggressive action of acid-peptic juices (Crawford, 1999).

Epidemiology

Peptic ulcer disease is a common problem worldwide, and the incidence in Thailand is the same as in western countries (Kachintorn *et al.*, 1992). In the United States, approximately 4 million people have peptic ulcer and 350,000 new cases are diagnosed each year (Crawford, 1999). Around 100,000 patients are hospitalized yearly, and about 3000 people die each year as a result of peptic ulcer disease.

Pathogenesis

Peptic ulcers appear to be produced by an imbalance between the gastroduodenal mucosal defense mechanisms (e.g. surface mucus secretion, bicarbonate secretion into mucus, mucosal blood flow, apical suface membrane transport, epithelial regenerative capacity and elaboration of prostaglandins) and the damaging forces (e.g. gastric acidity and peptic enzymes), as illustrated in Figure 1. Gastric acid and pepsin are requisite for all peptic ulcerations. Hyperacidity is not a prerequisite because only a minority of patients with duodenal ulcers have hyperacidity, and it is even less common in those with gastric ulcers. Gastric ulceration can readily occur when mucosal defenses fall, however, as when mucosal blood flow drops, gastric emptying is delayed, or epithelial restitution is impaired (Crawford, 1999).

Helicobactor pylori infection is present in virtually all patients with duodenal ulcers and about 70% of those with gastric ulcers.

Cigarette smoking impairs healing and favors recurrence and so is suspected of being ulcerogenic, possible by suppression of mucosal prostaglandin synthesis. Alcohol has not been proved to cause peptic ulceration directly, but alcoholic cirrhosis is associated with an increased incidence of peptic ulcers (Crawford, 1999). Additionally, corticosteroids in high dose and with repeated use have been implicated in promoting ulcers.

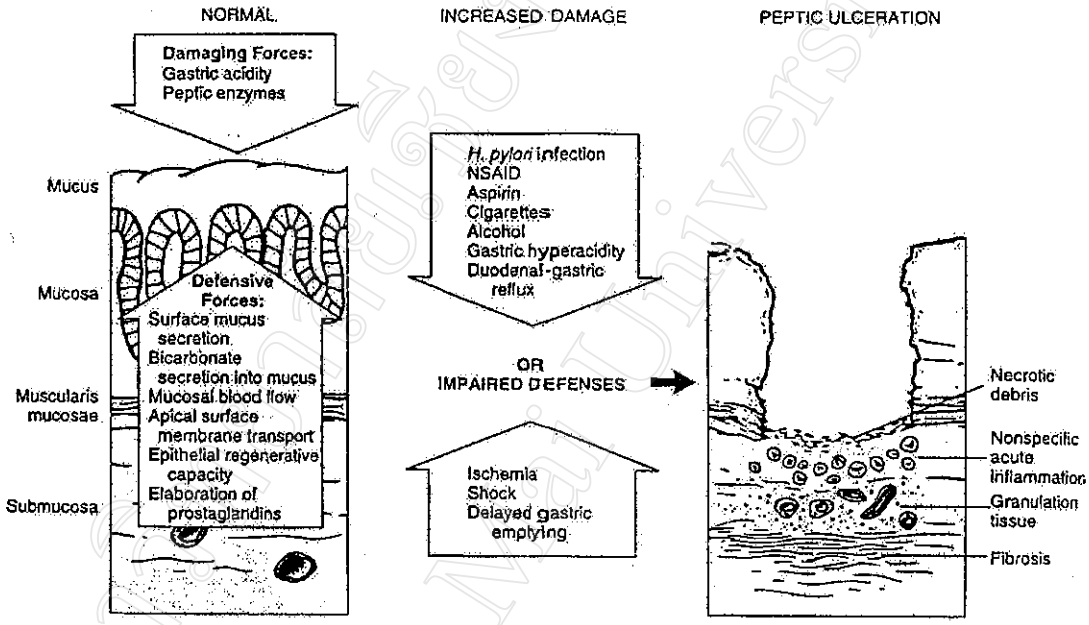

Figure 1 Diagram of aggravation causes of, and defense mechanisms against, peptic ulcer (from James M. Crawford: " The Gastrointestinal." in *Robbin: Pathologic Basis of Disease*, 6th ed. W.B. Saunders Co., USA, pp. 794, 1999.

Richardson (1989) and Friedman and Peterson (1998) described aggressive and defensive factors and their roles in peptic ulcer as follows:

1. Aggressive factors

1.1 Acid and pepsin: The gastric mucosal secretes acid by a process involving oxidative phosphorylation. Multiple chemical, neural, and hormonal factors participate in the regulation of gastric acid secretion. Acid secretion is stimulated by gastrin and by postganglionic vagal fibers via muscarinic cholinergic receptors on parietal cells.

Gastrin, the most potent known stimulant of gastric acid secretion, is contained in and released into the circulation from G cells. The release gastrin is stimulated by the neuropeptide, gastrin-releasing peptide and inhibited by somatostatin produced by D cells in the antrum. Gastrin stimulates gastric acid secretion by a direct stimulation of parietal cells and by stimulation of histamine release from enterochromaffin-like (ECL) cells.

Vagal stimulation increases gastric acid secretion by cholinergic stimulation of parietal cell secretion, by enhancing release of gastrin from antral G cells (by both inhibition of the release of somatostatin by antral D cells and by direct stimulation of G cells).

The gastric mucosal contains large amounts of histamine contained in cytoplasmic granules of mast cells and ECL cells. Histamine is the most important stimulant of gastric acid secretion and is released form ECL cells by the action of both gastrin and cholinergic activity. The basolateral membranes of parietal cells contain receptors for histamine, gastrin, and acetylcholine that stimulate acid secretion and for prostaglandins and somatostatin that inhibit acid secretion. Histamine stimulates gastric secretion by increasing parietal cell cyclic adenosine monophosphate (AMP), thereby activating cyclic AMP-dependent protein kinase.

The major physiologic stimulus for gastric acid secretion is ingestion of food. The regulation of gastric acid secretion has been classified into three phases: cephalic, gastric and intestinal. The cephalic phase, which includes cortical and hypothalamic components, is mediated primarily by vagal activation, which increases gastric acid secretion principally by direct stimulation of ECL and parietal cells and to a lesser extent by promoting gastrin release. The gastric phase results from stimulation of chemical and mechanical receptors in the gastric wall by luminal contents. Mechanical distention of the stomach stimulates gastric acid secretion but results in little gastrin release. Food (principally protein and products of protein) in the stomach promotes gastric acid secretion by increasing gastrin release. Food in the proximal small intestine stimulates the intestinal phase of gastric acid secretion by producing the release of small amounts of gastrin and other peptides that stimulate gastric secretion and by a direct effect of absorbed amino acids on parietal cells.

1.2 *Helicobactor pylori* infection: *H. pylori* produced a variety of proteins that appear to mediate or facilitate its damaging effect on the gastric mucosa. It reduces the thickness and viscosity of the mucous gel overlying the gastric mucosal epithelial cells.

1.3 Nonsteroidal anti-inflammatory drugs (NSAIDs): NSAIDs (e.g. aspirin, indomethacin, diclofenac, phenylbutazole, etc) associated with an increased incidence of gastric ulcer. NSAIDs are directly toxic to the gastric mucosa and they deplete protective endogenous mucosal prostaglandin (PG) by inhibiting PG synthesis. They also may contribute to ulcer formation by interrupting the gastric mucosal barrier, permitting back-diffusion of hydrogen ions that may injure the gastric mucosa. They reduce gastric mucus and bicarbonate secretion and may increase gastric acid secretion. Depletion of mucosal prostaglandins also impairs epithelial cell replacement after injury.

2. Defensive factors

2.1 Gastric mucus: Gastric mucus secreted by mucous cells of the gastric mucosal epithelium and gastric glands is important in mucosal defense and in preventing peptic ulceration. Mucus secretion is stimulated by mechanical or chemical irritation and by cholinergic stimulation.

2.2 Gastric bicarbonate: Nonparietal gastric epithelial cells secrete bicarbonate ions into the mucus gel. Gastric bicarbonate secretion stimulated by calcium, certain PGE and F series, cholinergic agents, and α-adrenergic agents.

2.3 Mucosal barrier: Normally, the luminal surfaces and intercellular tight junctions of the gastric epithelial cells create a gastric mucosal barrier that is almost completely impermeable to diffusion of hydrogen ions from the lumen. The mucosal barrier can be interrupted by bile acids, salicylates, ethanol and weak organic acids, permitting hydrogen ions to diffuse into gastric tissue. The results may be cell injury, release of histamine from mast cells, further stimulation of acid secretion, damage to small blood vessels, mucosal hemorrhage, and erosion or ulceration.

2.4 Mucosal blood flow: The maintenance of normal blood flow to the gastric mucosa is an essential component of mucosal resistance to injury. Decreased mucosal blood flow, accompanied by diffusion of luminal hydrogen ions, is thought to be important in producing gastric mucosal damage.

2.5 Prostaglandins: PGs are abundant in the gastric mucosa. Administration to animals of various PGs, particularly those of the E series, has been shown to prevent gastric mucosal injury caused by a wide variety of agents. Endogenous PGs stimulate secretion of gastric mucus and bicarbonate. They participate in the maintenance of gastric mucosal blood flow and of the integrity of the gastric mucosal barrier and promote epithelial cell renewal in response to mucosal injury.

Physiologic and pharmacological regulation of gastric secretion are diagrammatically shown in Figure 2 (Brunton, 1996)

Three major pathways regulating parietal acid secretion include 1) neural stimulation via the vagus nerve, 2) endocrine stimulation via gastrin release from antral G cells, and 3) paracrine stimulation by release of histamine from ECL cells.

Vagal stimulation and gastrin released from duodenal and antral G cells stimulate histamine release from paracrine ECL or mast cell. Histamine in turn activates the parietal cell H_2-receptor that is linked to the stimulation of adenylyl cyclase, causing activation of the cyclic AMP pathway. Gastrin and muscarinic stimuli also may act directly on the parietal cell to activate Ca^{+2} sensitive pathways. H_2-receptor antagonists not only block the effects of histamine but also blunt responses to acetylcholine and gastrin, thus contributing to the remarkable clinical efficacy of these agents.

Figure 2 provides a rationale for the modest inhibitory effects of anticholinergic agents, the impressive inhibition of acid secretion by H_2-receptor antagonists, and the effects of neutralizing gastric HCl with antacids on production and maintenance of gastric acidity. Covalent inhibitors of the H^+, K^+-ATPase, such as omeprazole, inhibit acid secretion, the final common pathway in gastric acid secretion. Prostaglandins, by inhibiting histamine-stimulation of adenylyl cyclase activity in the parietal cell, reduce activity through the histamine-evoked cyclic AMP-dependent pathway and thereby reduce acid secretion. Prostaglandins stimulate the secretion of mucus and bicarbonate by adjacent superficial epithelial cells, contributing to the cytoprotective effects of endogenous prostaglandins of the E series and to the protective effects of stable analogs of prostaglandins E_1, such as misoprostol. The importance of the tonic role of prostaglandins in cytoprotection is manifested by the ulcerogenic effect of nonsteroidal anti-inflammatoly drugs (NSAIDs) that inhibit prostaglandin synthesis. Bismuth, sucralfate, and carbenoxolone also enhance the cytoprotection afforded by the mucous layer.

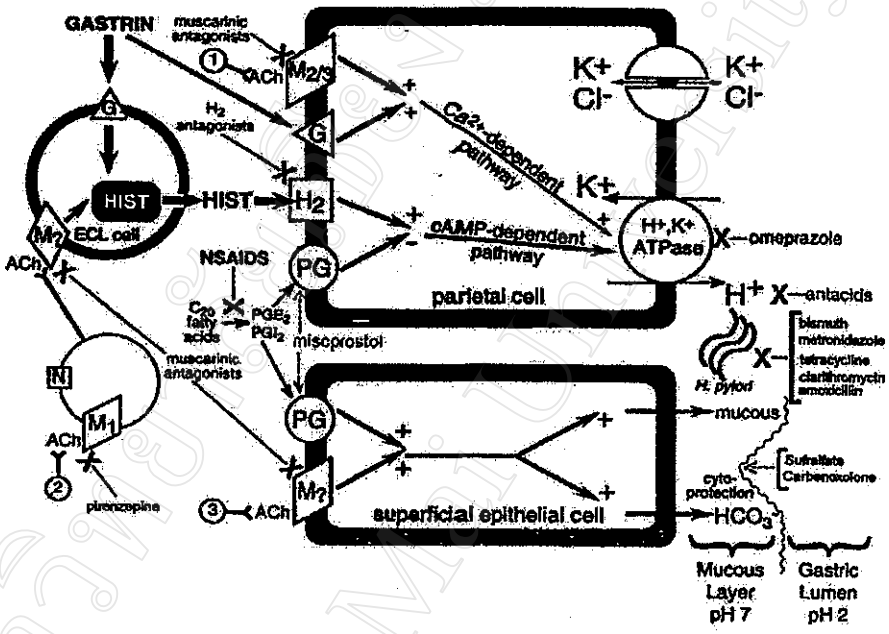

Figure 2 Physiological and pharmacological regulation of gastric secretion: the basis for therapy of peptic ulcer (from Laurence L. Bruton: "Agents for control of gastric acidity and treatment of peptic ulcers." in *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 9th ed. Macmillan Publishing Co., New York, pp. 902, 1996.)

Drugs for Treatment of Peptic Ulcers

The goals of therapy for ulcers are: relief from pain, promotion of healing, and prevention of recurrence. Therapeutic strategies are aimed at balancing aggressive factors (gastric acid secretion, pepsin, *H. pylori* infection) against defensive or cytoprotective factors (bicarbonate, mucous, and prostaglandin) (Brunton, 1996).

Medications used in peptic ulcers are grouped according to their therapeutic uses (Brunton, 1996; Altman, 1998) as follows:

1. Antacids

Gastric antacids are weak bases that react with gastric hydrochloric acid to form a salt and water. Their usefulness in peptic ulcer disease thus lies in their ability to reduce gastric acidity and, since pepsin is inactive in solution above pH of above 4.0, to reduce peptic activity.

Most antacids in current use are combinations of aluminum, magnesium, and calcium salts. The differences among antacids relate to the rapidity of their reaction with gastric acid, their neutralizing capability, their gastrointestinal side effects, and their systemic complication.

2. Gastric antisecretory drugs

2.1 Anticholinergic drugs (e.g. pirenzepine)

Anticholinergic drugs block the muscarinic effect of acetylcholine and so reduce the gastric secretion of acid.

2.2 Histamine H_2-receptor antagonists (e.g. cimetidine, ranitidine, famotidine, and nizatidine)

Histamine H_2- receptor antagonists are capable of over 90 % reduction in basal, food-stimulated, and nocturnal secretion of gastric acid after a single dose. Many trials have demonstrated their effectiveness in promoting the healing of duodenal and gastric ulcer and preventing their recurrence.

2.3 Proton Pump inhibitors (e.g. Omeprazole, Lansoprazole, Rabeprazole and Pantoprazole)

These agents inhibit the gastric parietal cell proton pump. They are effective for the short-term treatment of gastric and duodenal ulcers and for severe gastroesophageal reflux disease, and they are effective at reduce dosage for the prevention of recurrence of duodenal ulcer and esophagitis. They are superior to H_2 receptor antagonists and to misoprostol in the healing of NSAID-induced peptic ulcer. They are also used for pathologic hypersecretory syndromes such as Zollinger-Ellison syndrome, multiple endocrine neoplasias, and systemic mastocytosis. They produce only small and inconsistent changes in the volume of gastric juice and in the secretion of pepsin and intrinsic factor and do not affect gastric motility.

2.4 Octreotide

Octreotide: a synthetic somatostatin has been found to significantly inhibit the secretion of several circulating peptide hormones and to inhibit gastric and pancreatic secretion.

3. Mucosal protective agents

3.1 Carbenoxolone sodium

Carbenoxolone is a derivative of glycyrrhizic acid, which is found naturally in licorice root. It is readily absorbed from both the stomach and small intestine, and a large proportion absorbed from the stomach is bound to the gastric mucosa. The mechanism of action of carbenoxolone is not clear but is thought to involve an increase in the production, secretion and viscosity of intestinal mucus. It may relate to the lipophilic character of carbenoxolone (whereby it binds to membranes) and to its reported capacities to inhibit pepsin activity and to stimulate gastric glycoprotein synthesis.

3.2 Sucralfate

The mechanism of action of sucralfate is thought to involve selective binding to necrotic ulcer tissue, where it may act as a barrier to acid, pepsin, and bile. The drug has been shown to be effective in the healing of ulcers.

3.3 Colloidal bismuth compounds

These compounds also appear to work by selective binding to an ulcer and by coating it to protect the ulcer from acid and pepsin. They may also have some antimicrobial activity against *H. pylori.*

3.4 Prostaglandins (e.g. Misoprostol)

Prostaglandins E and I_2 (PGE and PGI_2), the predominant prostaglandins synthesized by the gastric mucosa, inhibit the secretion of acid and sitimulate the secretion of mucus and bicarbonate.

Misoprostol (PGE_1) is currently approved for the prevention of gastric ulcer disease induced by the administration of nonsteroidal anti-inflammatory drugs.

Although prostaglandins are produced by the gastric mucosa and are thought to have a cytoprotective effect (enhanced secretion of mucus and bicarbonate), the principal mechanism of action of prostaglandins appears to be the inhibition of gastric acid secretion.

4. Eradication of *Helicobacter pylori*

H. pylori is a gram negative rod that colonizes the mucus on the luminal surface of the gastric epithelium. *H. pylori* infection causes an inflammatory gastritis and is putative contributor to peptic ulcer disease, gastric lymphoma and adenocarcinoma. Single agent therapy of *H. pylori* infections has proven relatively ineffective. Double or triple antimicrobial therapies, in combination with antisecretory drugs (e.g. bismuth compound and metronidazole and amoxicillin or tetracycline), are being used successfully to treat peptic ulcers that are due at least in part to *H. pylori* infection.

Medicinal plants with anti-gastric ulcer activity

Turmeric (*Curcuma longa* Linn., Family Zingiberaceae)

The ethanolic extract of turmeric at the dose of 500 mg/kg orally showed a significant anti-ulcerogenic activity against gastric ulcerative induced by hypothermic restraint stress, pylorius ligation, indomethacin and reserpine administration (Rafatullah *et al.*, 1990). The gastric ulcer effect is proposed to be due to antisecretory as well as cytoprotective activity. However, it was revealed from a clinical study by Dau *et al.*, (1998), that turmeric at the dose of 6 g daily (as suggested in the Vietnamese pharmacopoeia) was not superior to placebo in healing duodenal ulcer either after four or eight weeks of treatment.

Ginger (*Zingiber officinale* Rosc., Family Zingiberaceae)

Ginger has cytoprotective and anti-ulcerogenic effects (Al-Yahya *et al.*, 1989). An extract of ginger at the dose of 500 mg/kg administered orally exerted highly significant cytoprotection against the injuries caused by 80% ethanol, 0.6 M HCl, 0.2 M NaOH and 25% NaCl. The extract also prevented the gastric ulcer induced by NSAIDs and hypothermic restraint stress. Additionally, terpenoid and 6-gingerol, the constituents from *Z. officinale*, showed anti-ulcer activity when tested in HCl/EtOH-induced gastric lesions in rats (Yamahara *et al.*, 1988).

Banana (*Musa sapientum* Linn., Family Musacese)

Various preparations of dried unripe plantain banana were found to be anti-ulcerogenic against aspirin-induced ulceration in the rats. The activity was suggested to be due to an ability to stimulate the growth of gastric mucosa, causing increases of mucosal mass and mucus production (Best *et al.*, 1984). Pulp powder of plantain banana was shown to have significant anti-ulcerogenic activity in rats subjected to aspirin, indomethacin, phenylbutazone and prednisolone treatment and in guinea-pigs treated with histamine (Goel, 1986).

Black Tea (*Camellia sinensis* Linn., Family Theaceae)

An oral administration of the hot water extract of black tea at the dose of 2 ml/100 g body weight per day for 7 days significantly reduced the incidence of ulcers in rats that were induced by aspirin, indomethacin, ethanol, reserpine and cold restraint stress (Maity *et al.*, 1995).

It was proposed from the further study of Maity *et al.*, (1998) that glutathione plays a major role as an endogenous antioxidant in cytoprotection against ulceration afforded by the black tea extract.

Plau-noi (*Croton sublyratus* Kurz., Family Euphorbiaceae)

Plaunotol is extracted from Plau-noi, a Thai medical plant and marketed as a cytoprotective antiulcer agent for gastritis and gastric ulcer in Japan (Miyoshi *et al.*, 1985). Plaunotol facilitated the healing of chronic ulcers in rats at oral doses of 10-30 mg/kg/day. It increased gastric blood flow, mucus secretion, bicarbonate secretion, prostaglandin synthesis and potentiated the gastric mucosal resistance whereas its influence on gastric secretion was minimal (Kobayashi and Tabata, 1992). Intrajejunal administration of plaunotol in three different doses (80, 160, and 320 mg/30 min) resulted in significant increases in both plasma secretin concentration and pancreatic bicarbonate secretion in a dose-related manner (Shiratori *et al.*, 1989).

Plaunotol also has a bactericidal effect against *Helicobacter pylori*, which may result from an interaction with the bacterial cell membrane. The mechanism by which plaunotol directly fluidizes and destroys the *H. pylori* membrane might make it difficult for this organism to develop resistance to plaunotol (Koga *et al.*, 1998).

Sacaca (*Croton cajucara* Benth., Family Euphorbiaceae)

Trans-Dehydrocrotonin, the major diterpene isolated from *Croton cajucara* Benth, at an oral dose of 100 mg/kg showed a significant antiulcerogenic effect on ulcers induced by hypothermic restraint stress, ethanol, and pylorus ligature and no significant changes in indomethacin-induced gastric lesions (Souza *et al.*, 1998). It had a significant anti-ulcer activity with no apparent toxicological effects.

Background of *Croton oblongifolius* Roxb.

Croton oblongifolius Roxb. (Family Euphorbiaceae), a medium-sized tree, is widely distributed throughout Thailand. It is commonly known as "Plao Yai", "Plao Luang" (Figure 3). It is a medium tree, about 8 m high. Leaf is simple, alternate, oblong, elliptic-oblong, ovate or lanceolate. Flowers are greenish yellow, and fruits are 3-valved capsules (Chuakul *et al.*, 1997).

In traditional medicines, various parts of the *C. oblongifolius* Roxb plant are used as follows:

Leaf	: as element tonic, and to treat pruritic rash
Flower	: as antihelmintic
Fruit	: to alleviate dysmenorrhea
Seed	: as purgative
Stem bark	: to promote digestion, and to treat dyspepsia
Stem	: to treat muscular pain
Wood	: as antihelmintic
Root	: as carminative, and to treat dysentery and allergic dermatitis

Other uses of *C. oblongifolius* are: for anti- diarrhea (Mudgal and Pal, 1980) and relief headache or migraine (Sabnis and Bedi, 1983).

Pharmacological activities of *C. oblongifolious* include anti-peristaltic (Bhakuni *et al.*, 1971), hypotensive (Bhakuni *et al.*, 1971; Mokkhasmit *et al.*, 1971b), histamine-like smooth muscle stimulation (Mokkhasmit *et al.*, 1971b).

In rats, the LD50 of 250 mg/kg was observed with an intraperitoneal injection (Bhakuni *et al.*, 1971) whereas the dose of 10 mg/kg was non toxic when given orally or subcutaneously (Mokkhasmit *et al.*, 1971a).

Figure 3 *Croton oblongifolius* Roxb. Family Euphorbiaceae (from Chuakul W.; Saralamp, P.; Paonil, W.; Temsirirkkul, R. and Clayton, T. *Medicinal Plants in Thailand* volume II. Bangkok: Amarin Printing and Publishing, 1997.)

Other reported biological activities of *C. oblongifolious* are: anti-snake venom (Selvanayahgam *et al.*, 1994; Ogiso *et al.*, 1981), nematocidal (Jain, 1989), and insecticidal (Nayar, 1955) activity.

Phytochemical study of *C. oblongifolious* revealed the presence of hyperoside, oblongifoliol, plaunol A, plaunol E, quercetin and rhamnetin (Subramanian *et al.*, 1971; Farnsworth *et al.*, 1969; Ogiso *et al.*, 1981). Recently, four new labdane diterpenoids (Roengsumran *et al.*, 1999) and two cembranoids (Roengsumran *et al.*, 1998) were isolated from the plant by Assoc. Prof. Dr. Roengsumran and his colleagues at the Department of Chemistry, Faculty of Science, Chulalongkorn University.

The labdane diterpenoids were screened for anti-gastric ulcer activity in rats, and it was found that labda-7,12(*E*),14-triene-17-oic acid (DS4, structure shown in Figure 4), showed good anti-gastric ulcer activity. When tested in the restraint water immersion stress-induced gastric ulcer model, at the dose of 50 mg/kg (given intraperitoneally), DS4 showed 97% inhibition, whereas cimetidine (a reference, H_2-antagonist) showed 44 % inhibition.

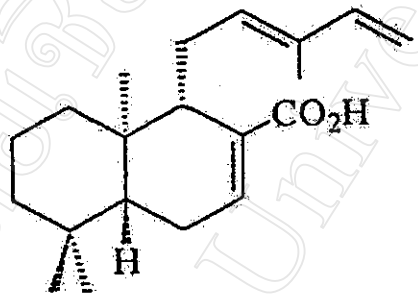

Figure 4 Structure of labda-7,12(*E*),14-triene-17-oic acid (DS4), a labdane diterpenoid from *Croton oblongifolius* Roxb

Purposes of the study

The present study was undertaken to study an anti-gastric ulcer activity of a labda-7,12(*E*),14-triene-17-oic acid (DS4) isolated from *C. oblongifolious*, according to following objectives

a) To assess the anti-gastric ulcer activity of DS4.
b) To compare the anti-gastric ulcer effect of DS4 to those of available reference drugs.
c) To study the possible mechanism (s) of action mediating anti-ulcer activity of DS4.