CHAPTER 1

INTRODUCTION

Peptic ulcer disease is commonly used to refer to ulcerations of the stomach, duodenum or both. Peptic ulcers can develop in any portion of the gastrointestinal tract that is exposed to acid and pepsin in sufficient concentration and duration (1). Ulcers are defined as a breach in the mucosa of the alimentary tract that extends through the muscularis mucosa into the submucosa or deeper (2).

Epidemiology of peptic ulcer disease

Peptic ulcer disease is a common problem in Thailand and throughout the world. In Thailand, the prevalence of gastric ulcers is approximately 10-20% of the population (3). Gastric ulcers usually occur in people ranging in age between 40-70 years old and found more frequently in men than women (4).

The pathophysiology of peptic ulcer disease

The pathophysiology of peptic ulcer disease is an imbalance between mucosal defensive factors such as bicarbonate, mucus, prostaglandin, nitric oxide and other peptides and aggressive factors especially acid and pepsin (5). Additionally, *Helicobacter pylori* and exogenous agents such as nonsteroidal anti-inflammatory drugs (NSAIDs) interact in complex ways to cause an ulcer.

Defensive factors

Gastric mucus

The mucus consists of mucins (glycoproteins) and water and is secreted by the surface epithelial cells and the mucus neck cells. Mucus is an insoluble gel that coats the mucosal surface of stomach, slow ion diffusion and prevents mucosal damage by acid, pepsin and other chemicals (5).

Gastric epithelium

Intercellular tight junctions of the gastric epithelial cell provide a barrier to the back-diffusion of hydrogen ions. When epithelial disruption occurs, restitution by cells migration along the basement membrane to fill in the defects and restores epithelial barrier integrity is rapidly followed (2).

Bicarbonate

Bicarbonate is secreted by superficial gastric epithelial cells to neutralize the acid in the region of mucosal cells. It raises pH and prevents acid mediated damage (5).

Prostaglandins

Prostaglandins are synthesized by gastric mucosa and play role in gastric epithelial defense**.** They can prevent gastric injury by cytoprotective effect that include stimulation of mucus and bicarbonate, inhibition of acid secretion by parietal cells and enhancement of mucosal blood flow and epithelial cell restitution (6).

Gastric mucosal blood flow

Blood flow plays important roles in protecting gastric mucosa and healing mucosal damage. Blood flow provides much of the energy and the substrates necessary for maintaining epithelial cell integrity and for effecting protective epithelial cell functions such as mucus production and bicarbonate secretion. Blood flow also removes acid that diffuses through an injured mucosa and transports the bicarbonate to the surface epithelial cells, a process that appears to against acid-peptic injury during acid secretion by the stomach (1).

Nitric oxide

Several studies suggest that endogenously produced NO increases the mucosal blood flow and stimulates gastric mucus secretion (7, 8, 9). Inhibition of nitric oxide synthesis dose-dependently induced acute mucosal damage, and hemorrhagic necrosis (10).

Gastrointestinal peptides

Several gastrointestinal peptides show inhibition of gastric acid secretion including somatostatin, vasoactive intestinal peptide (VIP), secretin, cholecystokinin (CCK), glucagon, gastric inhibitory polypeptide (GIP), calcitonin gene-related peptide (CGRP), nuerotensin and enkephalin (11). Somatostatin is a potent inhibitor of

gastric acid secretion via inhibition of histamine release from gastric enterochromaffin-like cells (12, 13). VIP stimulates pancreatic bicarbonate secretion, and inhibits gastrin-stimulated gastric acid secretion. It also prevented stress-induced ulcers, inhibited mast cell degranulation and protected gastric tissue from lipid peroxidation (14). GIP acts at the parietal cell to inhibit histamine-stimulated acid secretion (15). The function of secretin is to promote the secretion of pancreatic and biliary HCO_3^-. Secretin also inhibits the effects of gastrin on the parietal cells (16). CCK inhibited gastric acid secretion in rats by activation of type A cholecystokinin receptors and through release of endogenous somatostatin (17). The inhibitory effect of neurotensin on gastric acid secretion is dependent on vagal innervation of the parietal cell (18).

Aggressive factors

Hydrochloric acid secreted by the parietal cell

The stimulation of acid secretion resulting from the ingestion of food can be divided into 3 phases: cephalic, gastric and intestinal phases. The cephalic phase involves the central nervous system. Tasting, smelling, sighting, chewing and swallowing food stimulate gastric acid secretion by send nerve impulses via vagus nerve to the parietal cell and G cells. The gastric phase is a result of gasric distension and chemical agents. When the stomach is distended, mechanoreceptors and the neural are stimulated, which release acetylcholine (ACh). ACh stimulates G cells or peptides and amino acids act directly on G cells to release gastrin, which stimulates parietal cells secrete gastric acid. During the intestinal phase protein digestion products in duodenum stimulate gastric acid secretion. Distension of the small intestine, probably via the release of the hormone enterooxyntin from intestine endocrine cells, stimulates acid secretion (19).

The regulation of gastric acid secretion by parietal cells is important in peptic ulcer. Parietal cells produce gastric acid (hydrochloric acid) in response to histamine acetylcholine and gastrin.

Acetylcholine (ACh) released from vagus nerve innervating the gastric mucosa, acts directly muscarinic receptor (M_3) on parietal cells. Vagal stimulation, increases the release of histamine and gastrin (5).

Histamine is released from the enterochromaffin-like (ECL) cells. The rate of formation and secretion of hydrochloric acid by parietal cells is directly related to the amount of histamine secreted by ACh released from vagus nerve and hormonal gastrin (20).

Gastrin is a hormone synthesized in endocrine cells of the mucosa of gastric antrum and duodenum. Its main action is stimulation of the secretion of acid by parietal cell. Gastrin is indirectly pepsinogen secretion (21).

The mechanism of HCl secretion is depicted in Figure 1. HCl secretion by the parietal cell is formed in the following manner: Hydrogen ions are formed from the dissociation of water molecules. The enzyme carbonic anhydrase catalyzes CO_2 and H_2O to HCO_3^- and H^+. HCO_3^- is exchanged for a chloride (Cl^-) on the basal side of the cell and the HCO_3^- diffuses into the blood and Cl^- diffuse into the lumen of stomach. H^+ is pumped out of the cell into the lumen of stomach in exchange for K^+, via the H^+/K^+-ATPase (proton pump) (16).

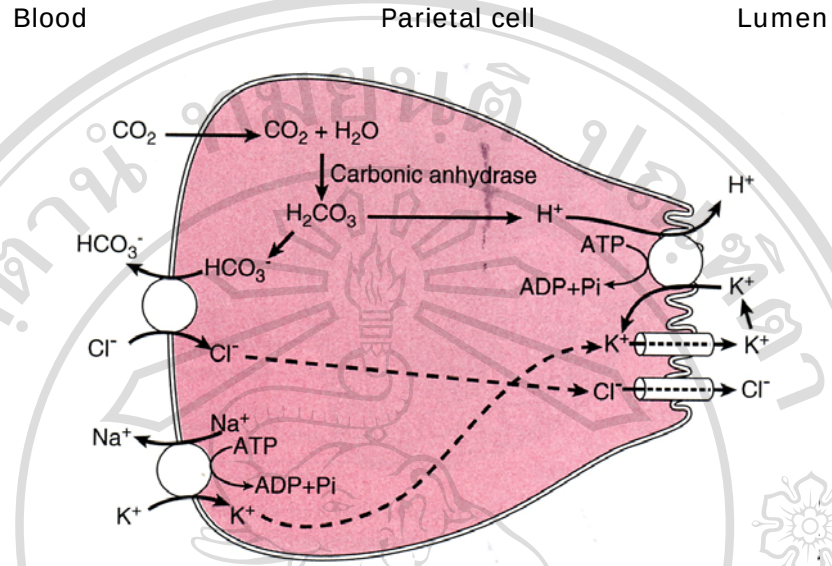

Figure 1 The mechanism of HCl secretion by the gastric parietal cell (from Medical Physiology. 2nd ed. Baltimore: Lippincott Williams and Wilkins; 2003, p. 486)

Pepsin

Pepsinogen is an inactive precursor of pepsin and released by the chief cells in gastric mucosa. When pepsinogen contacts with hydrochloric acid, it is activated to form active pepsin. Gastrin hormone and the vagus nerve trigger the release of both pepsinogen and HCl when food is ingested. Pepsinogens were divided into 2 main groups: I and II. Serum pepsinogens I is raised above the normal range in patients with peptic ulcer (22, 23). The relationship between peptic ulcer and pepsin secretion has been reported. Pepsin I was increased in patients with peptic ulcer disease and increased total pepsin secretion has an important role in the pathogenesis and reduced healing rates in peptic ulceration (24).

Nonsteroidal anti-inflammatory drugs (NSAIDs)

There is a 10-20% prevalence of gastric ulcers in long-term NSAIDs therapy (25). NSAIDs cause peptic ulcer by suppression of the cyclooxygenase isoforms leading to the decrease synthesis of prostaglandins. Prostaglandins play a critical role in maintaining gastroduodenal mucosal integrity and repair (6).

***Helicobactor pylori* infection**

H. pylori is a gram negative spiral bacterium. It enhances gastric acid secretion and impairs duodenal bicarbonate production, thus reducing luminal pH in the duodenum. Several studies suggested that duodenal ulcers and the large majority of gastric ulcers were associated with *H. pylori* infection (1).

Reactive oxygen species

Reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, hydroxyl radical involve in variety of tissue damage. Experimental studies suggest that ROS and lipid peroxidation play an important role in the pathogenesis of gastric ulceration induced by stress, ethanol and indomethacin (26-31).

Physiological and pharmacological regulation of gastric secretion

Physiological and pharmacological regulation of gastric secretion are shown in Figure 2. Gastic acid secretion is mediated through central (neutral) and peripheral pathways. Each pathway stimulates H^+ secretion by parietal cells. Neuronal (acetylcholine), paracrine (histamine), and endocrine (gastrin) factors all regulate acid secretion. Parietal cells have specific receptors (M_3, CCK_2, H_2) localized the

basolateral membrane. ACh release from postganglionic vagal fibers directly stimulates gastric acid secretion through muscarinic M_3 receptors. ACh also indirectly affects parietal cells by increasing the release of histamine from the enterochromaffin-like (ECL) cells and gastrin from G cells. Histamine diffuses via paracrine mechanism to the nearby parietal cells, where it activates H_2 receptors. Gastrin stimulates acid secretion indirectly by inducing the release of histamine by ECL cells and a direct effect on parietal cells also plays a lesser role. The histamine pathways act by increasing intracellular cAMP, whereas the ACh and gastrin pathway increase intracellular Ca^{2+} levels. Both cAMP and Ca^{2+} acts via protein kinases to activate H^+,K^+-ATPase (5).

Medicinal treatment of peptic ulcers usually consists of antacid to neutralize gastric acid or histamine H_2 receptor antagonist to inhibit acid secretion. The H^+,K^+-ATPase is inhibited by proton pump inhibitors such as omeprazole, the final step in acid secretion pathway. Prostaglandins bind to PGE_2 receptor (EP_3) and decreasing cyclic AMP and gastric acid. Prostaglandins, by inhibiting histamine-stimulated adenylyl cyclase activity in the parietal cell, reduce activity through the histamine-evoked cAMP-dependent pathway and thereby reduced acid secretion. Prostaglandins stimulate the secretion of cytoprotective mucus and bicarbonate on superficial epithelial cells. Misoprostol is a analog of PGE_1 also enhance mucosal defence. Sucrafate and carbenoxolone also enhance the cytoprotection afforded by the mucous layer (5).

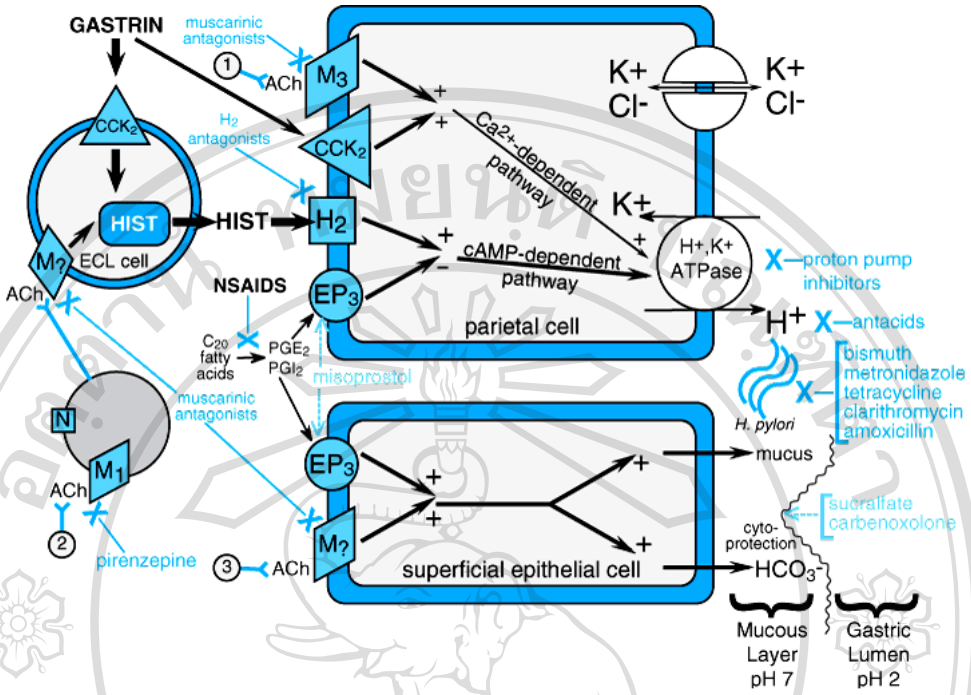

Figure 2 Physiological and pharmacological regulation of gastric secretion: the basis for therapy of peptic ulcer disease (from Goodman & Gilman's The pharmacological Basis of Therapeutics. 9th ed. New York: McGraw-Hill; 2007, p.968)

Drug for treatment of peptic ulcer

Drugs used in the treatment of acid-peptic disorders include peptic ulcer (gastric and duodenal), gastroesophageal reflux and stress-related mucosal injury may be divided into two classes (25).

1. Agents that reduce intragastric acidity: (5, 20, 25)

1.1 Antacids (e.g. sodium bicarbonate, calcium carbonate, magnesium hydroxide, aluminium hydroxide, etc.)

Antacids are weak bases that react with gastric hydrochloric acid to form salt and water. Antacids act by neutralizing gastric acid and thus raising the gastric pH. They have the effect of inhibiting peptic activity, which practically ceases at pH 5. Although their principal mechanism of action is reduction of intragastric acidity, they may also promote mucosal defense mechanism through stimulation of mucosal prostaglandin production.

1.2 H_2 receptor antagonists (e.g. cimetidine, ranitidine, famotidine, nizatidine, etc.)

The H_2 receptor antagonists inhibit acid secretion by reversible competing with histamine for binding to H_2 receptors on parietal cells. The H_2 receptor antagonists inhibit histamine, gastrin, acetylcholine stimulated acid secretion also reduced the volume of gastric secretion and pepsin secretion. Additionally, these agents not only decrease both basal and meal stimulated acid secretion but also promoting healing of duodenal ulcer.

1.3 Proton pump inhibitors (e.g. omeprazole, lansoprazole, rabeprazole, pantoprazole, etc.)

Proton pump inhibitors acts by irreversible inhibition of the H^+/K^+ ATPase (proton pump), the final step in acid secretion pathway. Proton pump inhibitors inhibit both basal and stimulated gastric acid secretion. They are used to promote healing of gastric and duodenal ulcers and to treat gastroesophageal reflux disease (GERD) and pathological hypersecretory conditions. In addition, they are also for treatment and prevention of recurrence of NSIADs associated gastric ulcers and reduce the risk of duodenal ulcer recurrence associated with *H. pylori* infections.

2. Mucosal protective agents: (5, 20, 25)

2.1 Sucralfate

Sucralfate is consist of alminium hydroxide and octasulfated sucrose. In an acid environment (pH < 4), it forms a viscous, sticky polymer that adheres to epithelial cells and ulcers for up to 6 hours. Sucralfate may offer an advantage over proton pump inhibitors and H_2 receptor antagonists for prophylaxis of stress ulcers. In vitro studies indicated that it can inhibit the action of pepsin.

2.2 Prostaglandin analogues (e.g. misoprostol)

Prostaglandin E and I_2 inhibit gastric secretion by binding to the EP_3 receptor on parietal cells and decreasing intracellular cAMP and gastric secretion.

Misoprostol is an analog of prostaglandin E_1. It has both inhibition of gastric acid and mucosal protective properties. The mucosal protective activity is mediated by an increase of mucosal blood flow, stimulation mucus and bicarbonate secretion. Misoprostol reduces the incidence of NSAIDs-induced ulcers to less than 3% and the incidence of ulcer complications by 50%.

2.3 Colloidal bismuth compounds (e.g. bismuth subsalicylate, bismuth subcitrate, bismuth dinitrate)

Bismuth compounds are used in combination regimens to treat H. pylori involvement in peptic ulcer. Other possible mechanisms of action include: probably coats ulcers, mucosal protective against acid and pepsin, stimulates prostaglandin synthesis and bicarbonate secretion.

2.4 Carbenoxolone

Carbenoxolone is derived from natural product found in liquorice root. It promotes the production of gastric mucus, which is a protective barrier in the stomach against acid and pepsin.

3. Eradication of *Helicobacter pylori*

H. pyroli is implicated in the production of gastric and duodenal ulcers. Many regimens for *H. pyroli* eradication have been purposed. A combination therapy of antisecretory drugs and other chemotherapeutic agents are used. For example, a proton pump inhibitor and clarithromycin 500 mg and metronidazole 500 mg or amoxicillin 1 g are given twice a day for 14 days (5).

The antisecretory drugs such as proton pump inhibitors and H_2-receptor antagonists are extensively used to control increased acid secretion and acid related disorders caused by stress, NSAIDs and *H. pylori*. However, clinical evaluation of these drugs showed that there are incidences of relapses and adverse effects.

Anti-gastric ulcer activity of plants

Many Thai medicinal plants have been found to exhibit anti-ulcer activity in experimental studies such as *Curcuma longa* (tumeric), *Croton sublyratus* (plau-noi), *Croton oblongifolius* (plau-yai), *Musa sapientum* (banana), *Zingiber officinale* (ginger).

***Curcuma longa* (tumeric)**

C. longa (tumeric) showed anti-ulcer activity in rat**.** An intraduodenal administration of curcumin, 5-20 mg/kg, inhibited gastric acid secretion in pylorus-ligated rats, and oral administration prevented ethanol-induced gastric mucosal lesions (32, 33). The ethanol extract of turmeric at the dose of 500 mg/kg produced significant anti-ulcerogenic activity in rats to hypothermic-restraint stress, indomethacin, reserpine and pyloruic ligation experiment. Clinical study of tumeric was found to be effective in peptic ulcers patients (34).

***Croton sublyratus* (plau-noi)**

Plaunotol, an acyclic diterpene isolated from *C. sublyratus*, which has been found to possess a broad anti-ulcer spectrum is clinically used in the treatment of peptic ulcers. This compound acts by enhancing mucosal defences such as gastric mucosal blood flow, gastric bicarbonate secretion and mucus secretion, due to increased prostaglandin content in the gastric mucosa (35). Moreover, it shows antibacterial activity against *Helicobacter pylori*, which is associated with peptic ulcers (36, 37).

***Musa sapientum* (banana)**

Anti-ulcerogenic activity of banana pulp has been reported against ulcers induced by histamine in guinea pigs and phenylbutazone, restraint stress, prednisolone, indomethacin (38-42) and aspirin in rats (43, 44).

***Zingiber officinale* (ginger)**

Powdered rhizome of ginger has been used as a traditional medicine for gastrointestinal complaints including in treating peptic ulceration. The cytoprotective and anti-gastric ulcer studies of ginger have been observed. At the dose of 500 mg/kg of ginger extract orally exert highly significant cytoprotection against 80% ethanol, 0.6M HC1, 0.2M NaOH, 25% NaCl, indomethacin, aspirin and hypothermic restraint stress.induced gastric ulcers (45).

Anti-gastric ulcer activity of freshwater algae

Villagers in northern Thailand consume freshwater algae, Kai (consisted of *Microspora floccosa* and *Cladophora glomerata*), to calm down gastric ulcer. Both algae showed anti-gastric ulcer activity when tested in rats with gastric ulcer induced by restraint water immersion stress, HCl/EtOH, indomethacin-induced gastric ulcer (46, 47).

Anti-gastric ulcer activity of marine algae

Seaweed or marine algae has long been used in food and traditional medicines in China, Japan and Korea. Some of marine algae show anti-gastric ulcer activity in experimental studies.

Gracilaria fisheri

An anti-gastric ulcer activity of *G. fisheri*, a red seaweed, has been observed. An aqueous extract of *G. fisheri* showed anti-gastric ulcer activity when tested in rats with gastric ulcer induced by restraint water immersion stress, irritating substances: HCl/EtOH and a NSAID drug: indomethacin (48).

Chlorella vulgaris

An oral administration of dry powder of *C. vulgaris*, a green alga, showed protective effects against gastric ulcer induced by water immersion restraint stress and cysteamine in rats (49).

Sargassum polycystum

The hot water extract of marine brown alga *S. polycystum* exhibited anti-gastric ulcer activity and improved the gastric mucosa antioxidant defense system against HCl-ethanol induced gastric mucosal injury in rats (50).

Background of *Ulva reticulata* Forsskal

U. reticulata is green seaweed of the Ulvaceae family (Division Chlorophyta). The *Ulva* species is known by the common name as sea lettuce (Figure 3). The color is light green to grass green. It is found floating near shore, often entangled with other seaweeds. The thallus is an irregular shaped flat blade up to a meter long. The blade becomes netlike appearance due to the presence of variable sized patches of cells which are destroyed by release of reproductive cells (51).

The *Ulva* species are very high in iron, as well as high in protein, iodine, aluminum and manganese. They also contain vitamin A, vitamin B1, vitamin C, sodium, potassium, magnesium, calcium, soluble nitrogen, phosphorous, chloride, silicon, rubidium, strontium, barium, radium, cobalt, boron and trace elements (52).

Polysaccharides represent around 38-54% of the dry marine algal matter (53). The sulfated polysaccharides are characteristic of the marine algae including green algae such as *Ulva* species (54). Sulfated polysaccharide from *U. pertusa* exhibited the antihyperlipidemic and antioxidant activity (55, 56, 57).

Pharmacological activities of *U. reticulata* have been reported, such as antihepatotoxicity activity (58), antioxidant activity (59) and hypotensive activity (60).

A preliminary study in rats suggested that *U. reticulata* might have an anti-gastric ulcer activity. An aqueous extract of *U. reticulata* at the dose of 500 mg/kg showed an inhibitory effect (74%) on restraint water immersion stress-induced gastric ulcers. Therefore, it is interesting to carry out an investigation on the anti-gastric ulcer activity of *U. reticulata* by employing various experimental models in order to examine the anti-gastric ulcer activity and possible mechanism(s) involved in the activity.

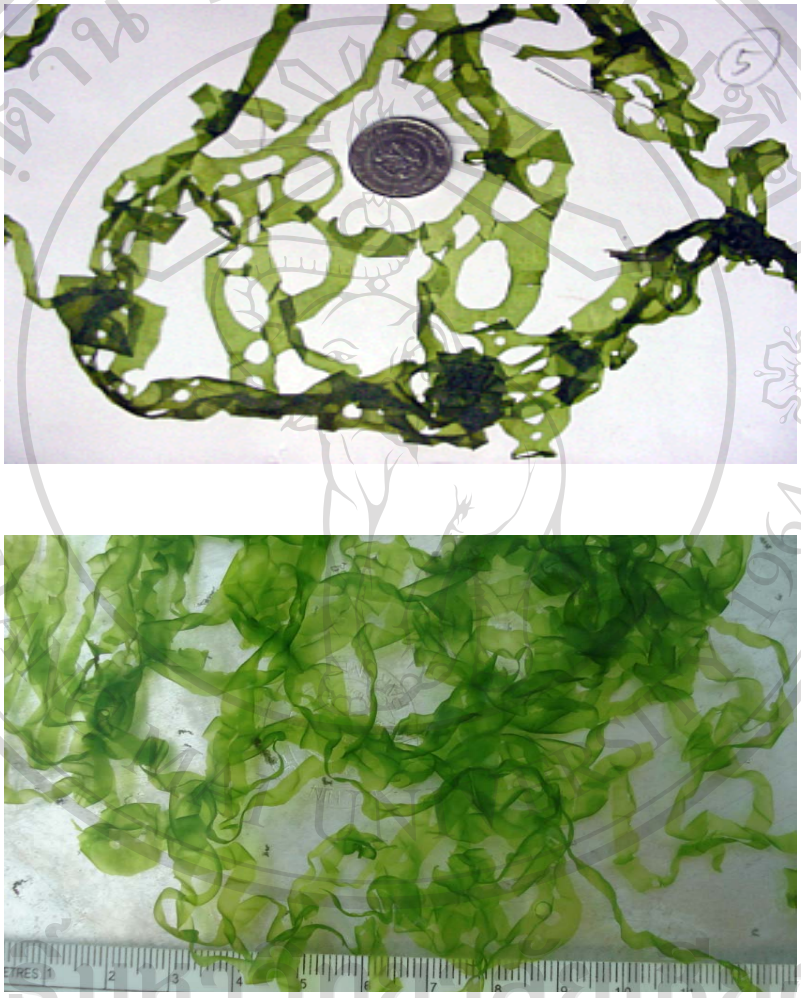

Figure 3 Marine alga: *Ulva reticulata* Forsskal

Purposes of the study

The purposes of the present study of the aqueous extract of *U. reticulata* were

1. to investigate an anti-gastric ulcer activity of *U. reticulata*
2. to study the possible mechanism(s) mediated the anti-gastric ulcer activity of *U. reticulata*