CHAPTER 4

DISCUSSION

Peptic ulcer has multiple etiopathogenesis. It is now believed that peptic ulcer results from an imbalance between defensive (cytoprotective) factors and aggressive factors. Defensive factors are bicarbonate, mucus, prostaglandin, nitric oxide and other peptides. Aggressive factors are acid, pepsin, *H. pylori*, ethanol and NSAIDs (5). Accordingly, drugs used in the treatment of peptic ulcer are known to possess the property of increasing the defensive factors or decreasing aggressive factors.

In the present study, the aqueous extract of *U. reticulata* at the doses of 100, 200 and 500 mg/kg exhibited anti-gastric ulcer activity on experimental models of which the gastric ulcers in rats were induced by different mechanisms including: the restraint water immersion stress, HCl/EtOH, indomethacin and histamine. In the pylorus ligated rats, the aqueous extract of *U. reticulata* at the doses of 200 and 500 mg/kg caused a decrease of total acidity and an increase of gastric pH. Similarly to cimetidine, the aqueous extract of *U. reticulata* could inhibit histamine induced increased heart rate when tested in the isolated guinea-pig atria experiment.

The restraint water immersion stress-, HCl/EtOH-, indomethacin-induced gastric ulcers in rats are models commonly used for evaluation of anti-gastric ulcer activity. Various mechanisms involved in the gastric ulcer production of these experimental models have been proposed.

In the restraint water immersion stress model, the gastric ulcers have been suggested to occur as a result of multifactorial impairment of mucosal defense system (69). Vagal overactivity is suggested to be the main factor in stress induced ulceration because stress-induced gastric ulcers in animal models may be partially or entirely prevented by vagotomy (70). Moreover, disturbance of gastric mucosal microcirculation (71), stimulation of vagal nerve resulting in increased gastric secretion (72), abnormal gastric motility (73), decreases of gastric mucosal blood flow

(74), gastric mucus production (75) and prostaglandin synthesis (76) have been considered for pathogenesis of stress-induced gastric ulcer. Reactive oxygen species (ROS) may play an important role in gastric ulceration induced by stress. ROS such as superoxide anion, hydrogen peroxide and hydroxyl radical are potent oxidizing agents and damage cellular membranes by lipid peroxidation being a major consequence (26, 27). Das *et al*. (26) found that restraint-cold stress in rats caused lipid peroxidation and hydroxyl radical formation.

The aqueous extract of *U. reticulata* significantly inhibited ulcer formation when tested in the restraint water immersion stress in rat model. Mechanisms such as inhibition of gastric acid secretion through cholinergic pathway, enhancement of gastric mucosal defensive factors including prostaglandins and antioxidant activity are likely to be involved its anti-gastric ulcer activity.

The HCl/EtOH-induced gastric ulcers model has been widely used for the evaluation of gastroprotective activity (77). HCl/EtOH-induced gastric ulcer is due to a direct necrotizing effect to gastric mucosa (78). HCl causes severe damage to gastric mucosa (79). It has been reported that a depression of gastric defensive mechanism plays roles in the formation of gastric mucosal ulcer by necrotizing agents such as ethanol (80). The depression of gastric defensive mechanisms include the reduction of mucus production, gastric mucosal blood flow, prostaglandins and endogenous glutathione (81). Ethanol can cause acute gastric mucosal injury by disturbing blood circulation in stomach causing gastric ischemia due to vasoconstriction (82, 83, 84). Additionally, the ability of ethanol to induce the formation of leukotriene C_4, a lipoxygenase derived metabolite of arachidonic acid has been reported (85). Ethanol-induced gastric ulceration in rats was found to be inhibited by leukotriene antagonist and 5-lipoxygenase inhibitor (86). Furthermore, it was found that ethanol-induced ulcer was inhibited by agents that enhance mucosal defensive factors such as prostaglandins (87). Robert *et al*. (88, 89) observed that administration of exogenous prostaglandins before absolute ethanol dose-dependently prevented the hemorrhagic gastric mucosal damage. Additionally, mild irritant given before a necrotizing agent (e.g absolute ethanol) showed a cytoprotective effect and this protection was suggested to be due to stimulating the release of endogenous prostaglandins (90). The property of agents that protect the gastric mucosa against

necrotinizing agents such as ethanol and HCl were defined as so called "cytoprotective" by Robert *et al*. ROS and lipid peroxidation play role in the pathogenesis of ethanol-induced gastric ulcers. Oxygen radicals have been directly implicated in the damage of cell membranes after administration of ethanol (91, 92). An intragastric administration of superoxide dismutase (a free radical scavenging enzyme) was able to protect the gastric mucosa against the damaging of ethanol (93).

The aqueous extract of *U. reticulata* significantly inhibited ulcer formation induced by HCl/EtOH, thus suggesting that it exerts a cytoprotective effect. It is possible that the cytoprotective effect of *U. reticulata* is mediated via an increase of gastric mucosal resistance by stimulating prostaglandin synthesis, inhibiting leukotriene synthesis and antioxidant activity.

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin are generally known to induce gastric ulcers by inhibition of biosynthesis of cytoprotective prostaglandins (e.g. PGE_2 and PGI_2) through cyclooxygenase (COX) pathway of arachidonic acid methabolism. The inhibition of COX pathway resulted in overproduction of leukotrienes and other products of 5-lipoxygenase pathway. Some leukotriene antagonists and 5-lipoxygenase inhibitors are capable to protect the gastric mucosa against lesions induced by oral or parenteral administration of NSAIDs (94). COX, the key enzyme for synthesis of prostaglandins, exists in two isoforms: COX-1 and COX-2. COX-1 is constitutively expressed in the gastrointestinal tract and has been suggested to maintain mucosal integrity and mucosal blood flow (95) whereas COX-2 is induced predominantly during inflammation (96, 97, 98). Prostaglandins have cytoprotective effect on gastric mucosa by stimulation of gastric mucus, and bicarbonate secretion, enhancement of mucosal blood flow, regulation of mucosal cell turnover and repair (25, 99). Lesch *et al*. (100) observed that higher amounts of prostaglandin E are detected at the site of ulceration than in non-ulcerated mucosa. Pretreatment with indomethacin (non-selective COX inhibitors) which significantly suppressed prostaglandin E generation, markedly delayed the healing of these lesions. Furthermore, NSIADs caused a reduction in gastric mucosal blood flow has been reported. Administration of indomethacin that inhibited prostaglandin formation reduces gastric mucosal blood flow in rats (101), conscious dogs (102) and in healthy subjects (103, 104). In

addition, Kawano *et al*. (105) reported that administration of indomethacin to healthy subjects caused reduction in the amount of hemoglobin in the gastric mucosa immediately, thus suggesting that the pathology of indomethacin induced acute gastric mucosal damage might be caused by ischemia. Yoshikawa *et al*. (31) found that the total area of gastric ulcers and concentration of lipid peroxides in the gastric mucosa increased after administration of indomethacin in rats. Furthermore, treatments with antioxidant enzymes (superoxide dismutase and catalase) could inhibit the increase of gastric mucosal lesions and lipid peroxides. The findings suggested that ROS and lipid peroxidation play an important role in pathogenesis of gastric mucosal injury induced by indomethacin.

The aqueous extract of *U. reticulata* showed anti-gastric ulcer activity when tested with indomethacin-induced gastric ulcers in rats, thus suggesting that it has cytoprotective effect. It is likely that the cytoprotective effect of *U. reticulata* could be mediated by increasing endogenous prostaglandin synthesis, inhibiting leukotriene synthesis and antioxidant activity.

Histamine-induced gastric ulcers in rat model is also used in the evaluation for anti-gastric ulcer activity. Histamine produces gastric ulceration by enhancing gastric acid secretion via H_2-receptor on parietal cells (5). The aqueous extract of *U. reticulata* extract was found to inhibit gastric ulcer formation induced by histamine. It is suggested that anti-gastric ulcer activity of *U. reticulata* may be due to inhibition of gastric acid secretion through H_2-receptor.

Comparison of anti-gastric ulcer activity of the aqueous extract of *U. reticulata* (at the dose of 500 mg/kg) when tested in 4 different models revealed that *U. reticulata* is highly effective in histamine-, HCl/EtOH- and indomethacin-induced gastric ulcers models with the percent inhibition of 93.67, 93.31 and 89.67, respectively. In the restraint water immersion stress model, the percent inhibition was 74.73.

Gastric acid is an important factor for the formation of gastric ulcer in pylorus-ligated rats (65). Pylorus ligation causes accumulation of gastric acid which lead to auto-digestion of the gastric mucosa and break down of the gastric mucosal barrier (106, 107). The aqueous extract of *U. reticulata* caused significantly decrease of total

acidity and increase of gastric pH after pylorus ligation for 5 h. The obtained result suggests that the aqueous extract of *U. reticulata* possesses an anti-secretory effect.

The aqueous extract of *U. reticulata* showed anti-gastric ulcer activity causing inhibition of histamine-induced gastric ulcers and exhibited antisecretory effect when tested in pylorus ligated rats. These effects may be mediated via antagonism at histamine H_2-receptor or muscarinic M_3-receptor or gastrin CCK_2-receptor.

Black *et al*. (68) first defined the H_2-receptor and characterized of H_2-receptor antagonists by performing the study using the isolated guinea-pig right atria. The actions of histamine such as stimulation of gastric acid secretion and increased heart rate were not antagonized by H_1-receptor antagonist but are competitively antagonized by imidazole derivatives and cimetidine (108). Similarly to cimetidine (H_2-receptor antagonist), the aqueous extract of *U. reticulata* could inhibit histamine induced increased heart rate of the isolated guinea-pig right atria. The regression lines of concentration-response (inhibitory effect on histamine induced increased heart rate) of the aqueous extract of *U. reticulata* and cimetidine were found to be parallel. Therefore, it is suggested that the aqueous extract of *U. reticulata* shares similar mechanism with cimetidine by antagonism at histamine H_2-receptor.

Robert *et al*. (88) coined the term “cytoprotection” to describe the property of prostaglandins and other compounds which have no structural similarity with prostaglandins by protecting gastric mucosal tissue from various necrotinizing agents such as ethanol, strong acids or bases, and NSAIDs like indomethacin and aspirin. The aqueous extract of *U. reticulata* showed a cytoprotective effect when tested against HCl/EtOH as well as indomethacin-induced gastric ulcers in rats. The cytoprotective effect is probably due to prostaglandins synthesis leading to stimulation of mucus and bicarbonate secretion and an increase of mucosal blood flow.

The gastric wall mucus plays an important role as a defensive factor against gastrointestinal damage. Further study was then carried out to determine the effect of the aqueous extract of *U. reticulata* on gastric wall mucus content of rats with HCl/Ethanol-induced gastric ulcers. The mucus consists of mucin-type glycoproteins which can be detected by amounts of alcian blue binding (109). Oral administration of HCl/EtOH resulted in a decrease amount of gastric wall mucus. In HCl/EtOH-

induced gastric ulcers experiment, the amount of gastric wall mucus of *U. reticulata* group was not significant different from that of control group. Thus pretreatment with the aqueous extract of *U. reticulata* could not protect the loss of gastric wall mucus caused by HCl/EtOH or increase gastric mucus secretion.

The cell walls of seaweeds are rich in matrix polysaccharides of different shapes and with different biological properties. The polysaccharide families in *Ulva* species consist of water-soluble ulvan, insoluble cellulose, peculiar alkali-soluble linear xyloglucan and a glucuronan (53). The water-soluble polysaccharide ulvan has viscosity and gelling characteristics (110, 111). The aqueous extract of *U. reticulata* is capable of forming gel which can coat the gastric mucosa as protective barrier against aggressive factors (such as HCl/EtOH) or bind to ulcers tissue.

ROS and lipid peroxidation play roles in the pathogenesis of gastric ulceration induced by stress, ethanol and indomethacin. The aqueous extract of *U. reticulata* was found to possess an antioxidant activity when tested in DPPH assay (112). Therefore it is possible that the antioxidant acivity of *U. reticulata* contributes to its anti-gastic ulcer activity.

The present study has shown that the aqueous extract of *U. reticulata* exhibit an anti-gastric ulcer activity when evaluated in various gastric ulceration models in rats including the restraint water immersion stress-, HCl/EtOH-, indomethacin- and histamine-induced gastric ulcers. The anti-gastric ulcer activity of *U. reticulata* is mediated via an inhibition of gastric acid secretion (anti-secretory effect) which is related to H_2-receptor antagonism property. The aqueous extract of *U. reticulata* has been shown to have an antioxidant activity which might play role in its anti-gastric activity. Results from the HCl/EtOH, indomethacin experiments suggest that the aqueous extract of *U. reticulata* possesses a cytoprotective effect. In the present work *U. reticulata* was prepared as an aqueous extract, thus the water soluble components in the extract might be responsible for exerting various mechanisms such as cytoprotective, H_2-receptor antagonist and antioxidant which mediate its anti-gastric ulcer activity.