DISCUSSION

The peptic ulcer results from an imbalance between aggressive factors and the maintaining of the 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, the methanolic extract of *K. galanga* showed anti-ulcer activity when evaluated in various experiment models, which included EtOH/HCl-, restraint water immersion stress-, pylorus ligation-, and indomethacin-induced gastric lesions. Reference drugs used in the study were cimetidine and misoprostol.

The model EtOH/HCl-induced gastric lesion is generally used in the evaluation for anti-ulcer activity. HCl caused severe damage to gastric mucosa (Yamahara *et al.*, 1988). Indeed, ethanol produced necrotic lesions in the gastric mucosa by its direct toxic effect reducing defensive factors, the secretion of bicarbonate and production of mucus (Marhuenda *et al.*, 1993). Development of ethanol-induced gastric hemorrhagic lesions

has been shown to be preceded by an early vascular damage and increases mucosal microvascular permeability (Guth *et al.*, 1984; Ohya and Guth, 1988 and Takeuchi *et al.*, 1989). The methanolic extract of *K. galanga* showed anti-ulcer activity causing a reduction of ulcer formation induced by EtOH/HCl, and the activity was dose related (Table 2). The methanolic extract of *K. galanga* and cimetidine at the dose of 100 mg/kg show anti-ulcer activity, and percent inhibitions of gastric ulcer of the former and the later were 91 and 82, respectively. It has been proposed that drugs which are effective against EtOH/HCl induced gastric lesions possess a gastric mucosal membrane protection action (Konturex *et al.*, 1984 and Lange *et al.*, 1985). It is therefore possible that *K. galanga* protects gastric wall mucus against EtOH/HCl damage probably by increasing mucosal resistance or potentiation of defensive factors and/or decreased aggressive factors.

Restraint water immersion stress-induced gastric ulcer has been widely used experimentally for the evaluation of anti-ulcer activity in rats because of data reproducibility (Murakami *et al.*, 1985). Disturbance of gastric mucosal microcirculation (Guth, 1972), alteration of gastric secretion (Kitakawa *et al.*, 1979) and abnormal gastric motility (Watanabe, 1966) have been considered to be the pathogenic mechanisms responsible for

stress-induced gastric mucosal lesions and gastric mucous depletion (Koo *et at.*, 1986). Although the mechanism of stress-induced ulceration is not clearly known, it is suggested to be mediated through histamine release with an enhancement in acid secretion from parietal cells and reduction in mucous production (Brodie and Hanson, 1960; Murakami *et al.*, 1985; Minano *et al.*, 1987). Many endogenous substances are known to affect HCl secretion by the parietal cell (Figure 14). Acetylcholine from the vagus nerve, histamine from mast cells, and gastrin from G cells, all stimulate the parietal cells directly to secrete HCl (Bullock and Boyle, 1995). Moreover, stress-induced ulcers can be prevented partly or entirely by vagotomy; therefore, vagal over activity has been suggested as the effector in stress-induced ulceration (Brodie and Hanson, 1960). 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 glucocorticoid level. Glucocortocoids can produce peptic ulcers probably by reducing the formation of PGE_2 and PGI_2 leading to a decreased mucous secretion in the stomach (Ganong, 1997). They probably enhance the recurrent rate of chronic peptic ulcers and the extent of the lesion by retarding the healing process.

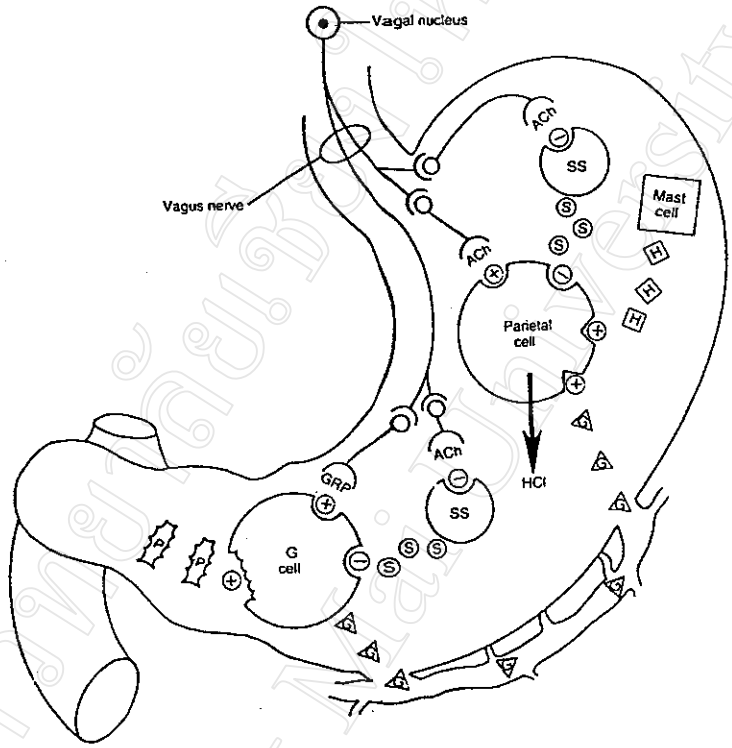

Figure 14 Endogenous substances affecting hydrochloric acid (HCl) secretion by the parietal cell. Acetylcholine (ACh) from vagus nerve, histamine (H) from mast cells, and gastrin (G) from G cells all stimulate the parietal cell directly to secrete HCl. The release of gastrin into the circulation, in turn, is stimulated by gastrin-releasing peptide (GRP) and protein digestion products (P). Somatostatin (S), released from somatostatin cells (SS), inhibits the release of both gastrin and HCl. Thus, the stimulation of vagal fibers, which causes the release of ACh and GPR but inhibits the release of somatostatin, has an amplified positive effect on parietal-cell HCl secretion. *Plus signs* = stimulation; *Minus signs* = inhibition. (Adapted from Johnson LR (ed): *Gastrointestinal Physiology*, 3rd ed. St. Louis, CV Mosby, 1985, pp. 72).

Similar to other studies (Brodie and Hanson, 1960; Maity *et al.*, 1995; Paiva *et al.*, 1998), the present study demonstrated the inhibitory activity of cimetidine against gastric ulcer induced by restraint water immersion stress. The methanolic extract of *K. galanga* significantly inhibited gastric ulcer-induced by restraint water immersion stress, but less effective than cimetidine. The methanolic extract of *K. galanga* at the dose of 100 mg/kg caused percent inhibition of 37, whereas cimetidine 100 mg/kg of 76 (Table 3). The anti-ulcer activity of *K. galanga* may be due to inhibition of gastric secretion (released by vagal activity), and increase of defensive factors. It is possible that *K. galanga* exerts anti-ulcer effect through an inhibition of gastric secretion (aggressive factor) and/or increase of the defensive factors. It was noted that among the percent inhibitions of ulcer formation caused by *K. galanga* in the experimental models used in the present study, the inhibition was lowest when tested in restraint water immersion stress induced gastric ulcer model (Figure 11).

The methanolic extract of *K. gala*nga was further tested in the pylorus ligation-induced gastric lesion model. This model is used for evaluation of anti-ulcer and anti-secretory activity (Shay *et al.*, 1945). Pylorus ligation caused accumulation of intraluminal HCl, leading to gastric mucosal damage.

Cimetidine exerted anti-ulcer and anti-secretory activity causing significant decreasing of both gastric volume and total acidity. The methanolic extract of *K. galanga* exhibited a significant preventive anti-ulcer effect (Table 4). *K. galanga* 100 mg/kg and cimetidine 100 mg/kg caused percent inhibitions of 62 and 91, respectively. It was also found that the extract failed to decrease total acidity, and that the methanolic extract of *K. galanga* at low dose (25 mg/kg) decreased gastric volume, whereas at high doses (50, 100 and 150 mg/kg) increased gastric volume (Table 5). The methanolic extract of *K. galanga* shows anti-ulcer activity, but is devoid of anti-secretory activity since it did not decrease gastric volume and total acidity. The anti-ulcer activity of *K. galanga* may possibly due to an increase of the defensive factors such as gastric mucus, bicarbonate and PGs.

NSAIDs, such as indomethacin are known to induce ulcer during the course of their anti-inflammatory action. NSAIDs inhibit cyclooxygenase (COX) leading to reduction of PG synthesis from arachidonic acid. Two isoforms of COX are COX-1, which is a constitutive enzyme, and COX-2, which is rapidly inducible and responsible for the production of pro-inflammatory PGs (Andrews and Goodman, 1998). High concentrations of PGs, especially PGE_2 and PGI_2, are present in

the normal gastric and duodenal mucosa and they are responsible for mucous production. Inhibition of PG synthesis by traditional NSAIDs such as indomethacin which non-selectively inhibit both COX-1 and COX-2, causes gastric and intestinal ulceration and delays gastric ulcer healing in chronic ulcer. Selective COX-2 inhibitors represent a new pharmacologic class of NSAIDs with minimal gastrointestinal toxicity. PGs, especially PGE_2, and their analogs inhibited the formation of gastric mucosal necrosis induced by such necrotizing agents including NSAIDs (Robert *et al.*, 1983).

Robert *et al.* (1979) coined the term 'cytoprotection' to describe the property of PGs (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. This pharmacological action is achieved independently of the inhibition of gastric acid secretion.

The methanolic extract of *K. galanga*, cimetidine and misoprostol significantly inhibited gastric ulcer induced by indomethacin (Table 6). *K. galanga* 100 mg/kg caused percent inhibition of 70 whereas cimtidine 100 mg/kg caused inhibition of 82%. The results suggest that anti-ulcer activity is probably

associated with increases of endogenous PGs and/or other defensive factors.

The effect of the methanolic extract of *K. galanga* on gastric wall mucus was studied in EtOH/HCl-induced gastric ulcer model. Cimetidine did not increase gastric wall mucus. On the contrary, a significant increase of gastric mucus was observed with *K. galanga* treatment (Table 7). It is indicated that the methanolic extract of *K. galanga* prevents EtOH/HCl-induced gastric damage by increasing mucous production.

The methanolic extract of *K. galanga* showed a spasmodic activity (increased ileal contraction), when tested in isolated guinea-pig ileal experiment (Table 8, Figure 12). The contraction induced by the extract was almost completely blocked by atropine, a cholinergic antagonist, but partly by chlorpheniramine, a histamine antagonist (Table 9, Figure 13). It is possible that cholinergic and histaminic mechanisms contribute to the spasmodic effect of *K. galanga*.

Gastric secretion is mediated through a cholinergic pathway (Ganong, 1997). *K. galanga*, which has been shown to have a cholinergic activity, therefore stimulates gastric secretion or has a gastric secretory activity. This activity is confirmed in the pylorus ligation experiment.

CONCLUSION

K. galanga significantly inhibited gastric ulcer formation induced by EtOH/HCl, indomethacin and stress. It is likely that *K. galanga* exerted anti-ulcer activity via defensive mechanism by increasing gastric mucus and bicarbonate secretion, and PG production. Accordingly, it is possible that *K. galanga* possesses a cytoprotective activity. In addition, *K. galanga* can increase mucus when it tested in EtOH/HCl-induced gastric ulcer model. It is suggested from the restraint water immersion stress-induced gastric ulcer model, that *K. galanga* probably has an anti-secretory activity. However, the pylorus ligation model clearly demonstrates that *K. galanga* has no anti-secretory activity. In isolated guinea-pig ileal experiment, *K. galanga* shows cholinergic activity, thus excluding an anticholinergic mechanism participating in anti-ulcer activity of *K. galanga*. Furthermore, cholinergic activity of *K. galanga*, might find to be useful for relieving dyspepsia due to peptic ulcer.