CHAPTER 3

RESULTS

The anti-gastric ulcer activity and the possible mechanism(s) involved in the activity of the aqueous extract of *U. reticulata* were investigated in rats by employing various experimental models, which included 1) restraint water immersion stress, 2) HCl/EtOH, 3) indomethacin, 4) histamine-induced gastric ulcers, 5) pylorus ligation, 6) determination of gastric-wall mucus of HCl/EtOH-induced ulceration and 7) isolated guinea pig right atria. The aqueous extract of *U. reticulata* at the doses of 100, 200, and 500 mg/kg were tested. Cimetidine (H_2-receptor antagonist) was used as a reference drug and given at a dose of 100 mg/kg.

Restraint water immersion stress-induced gastric ulcers in rats

Restraint water immersion stress induced hemorrhagic form of lesion in the glandular part of the stomach. The effect of the aqueous extract of *U. reticulata* on gastric ulcers induced by restraint water immersion stress is demonstrated in Figure 13. The aqueous extract of *U. reticulata* showed a dose related anti-gastric ulcer activity. The formation of gastric ulcer was reduced by the pretreatment with the aqueous extract of *U. reticulata* or cimetidine. Table 1 illustrates the ulcer index and percent inhibition of groups pretreated with the aqueous extract of *U. reticulata* and cimetidine. Statistically significant decrease of ulcer indexes of 5.75, 3.91 and 2.46 mm were found at the doses of 100, 200 and 500 mg/kg, respectively, whereas that of the control group was 9.53 mm. Percent inhibition of ulcer formation at doses of 100, 200, 500 mg/kg were found to be 39.66, 58.97 and 74.19, respectively. Cimetidine exhibited anti-ulcer activity, causing significant decrease of ulcer index to 0.51 mm and percent inhibition of 96.75.

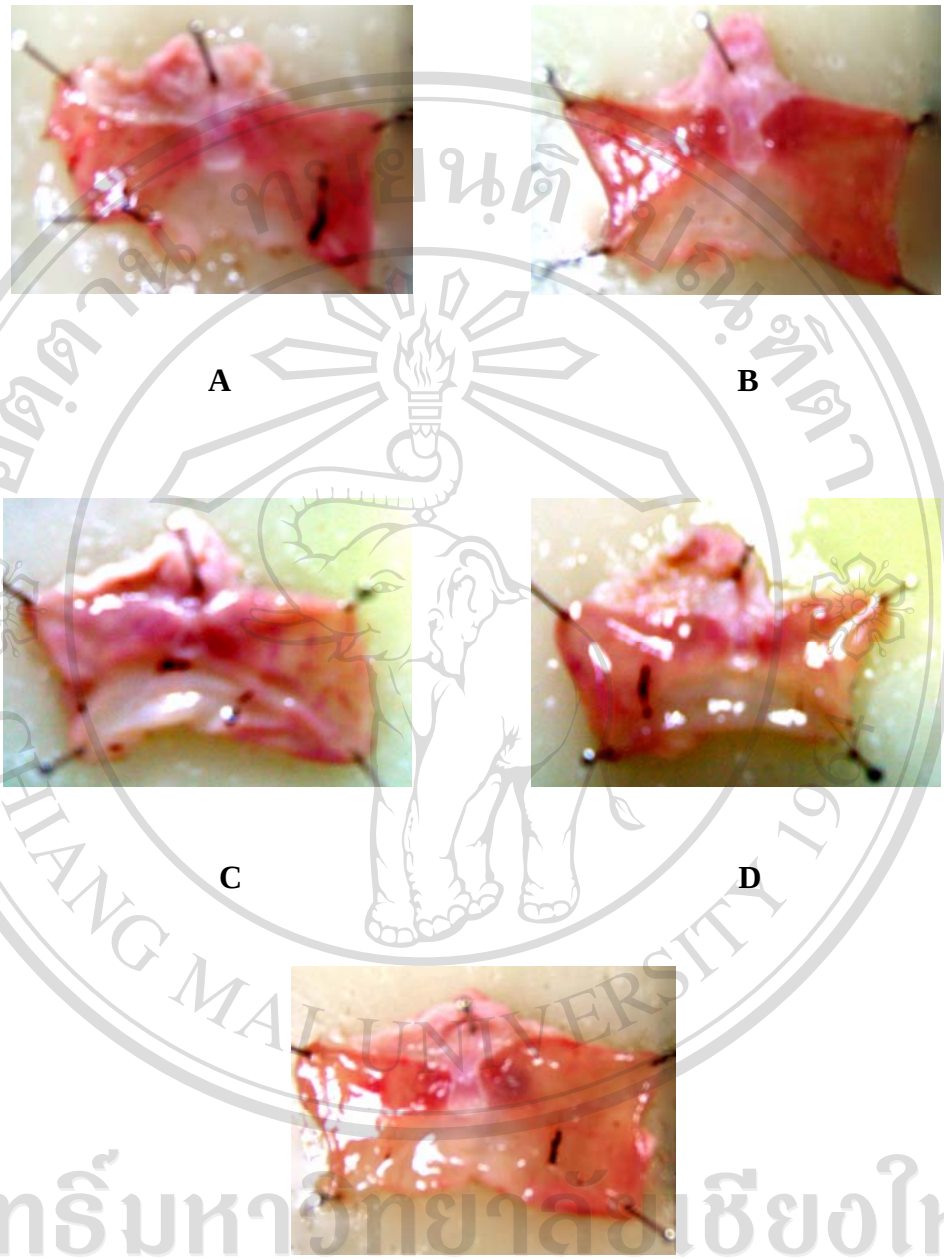

Figure 13 Gastric ulcers induced in rats by restraint water immersion stress: A) control, B) cimetidine 100 mg/kg, C) *U. reticulata* 100 mg/kg, D) 200 mg/kg and E) 500 mg/kg

Table 1 Effect of the aqueous extract of *U. reticulata* on restraint water immersion stress-induced gastric ulcers in rats

Group	Ulcer index (mm)	Inhibition (%)
Control	9.53 ± 1.14	-
Cimetidine		
100 mg/kg	0.51 ± 0.22 ***	96.75
U. reticulata		
100 mg/kg	5.75 ± 1.22 **	39.66
200 mg/kg	3.91 ± 0.89 ***	58.97
500 mg/kg	2.46 ± 0.58 ***	74.19

Data expressed as mean ± S.E.M. (n = 8)

Significantly different from control group: (** $p < 0.01$, *** $p < 0.001$)

HCl/EtOH-induced gastric ulcers in rats

An oral administration of HCl/EtOH to fasted rats resulted in severe gastric mucosal damage. In the control group clearly hemorrhagic elongated bands in the glandular segment of stomach were observed (Figure 14). The rats received pretreatment of the aqueous extract of *U. reticulata* or cimetidine had less gastric lesions than the control group. Table 2 shows the effect of the aqueous extract of *U. rcticulata* and cimetidine on HCl/EtOH-induced gastric ulcers in rats. The aqueous extract of *U. reticulata* only at the dose of 500 mg/kg that showed significant effect with the ulcer index was 6.05 mm, whereas that of the control group was 90 mm. Percent inhibition of ulcer formation of the aqueous extract of *U. reticulata* at the dose of 500 mg/kg was found to be 93.31. Cimetidine significantly inhibited the ulcer formation, showing an ulcer index of 29.50 mm and percent inhibition of 67.37.

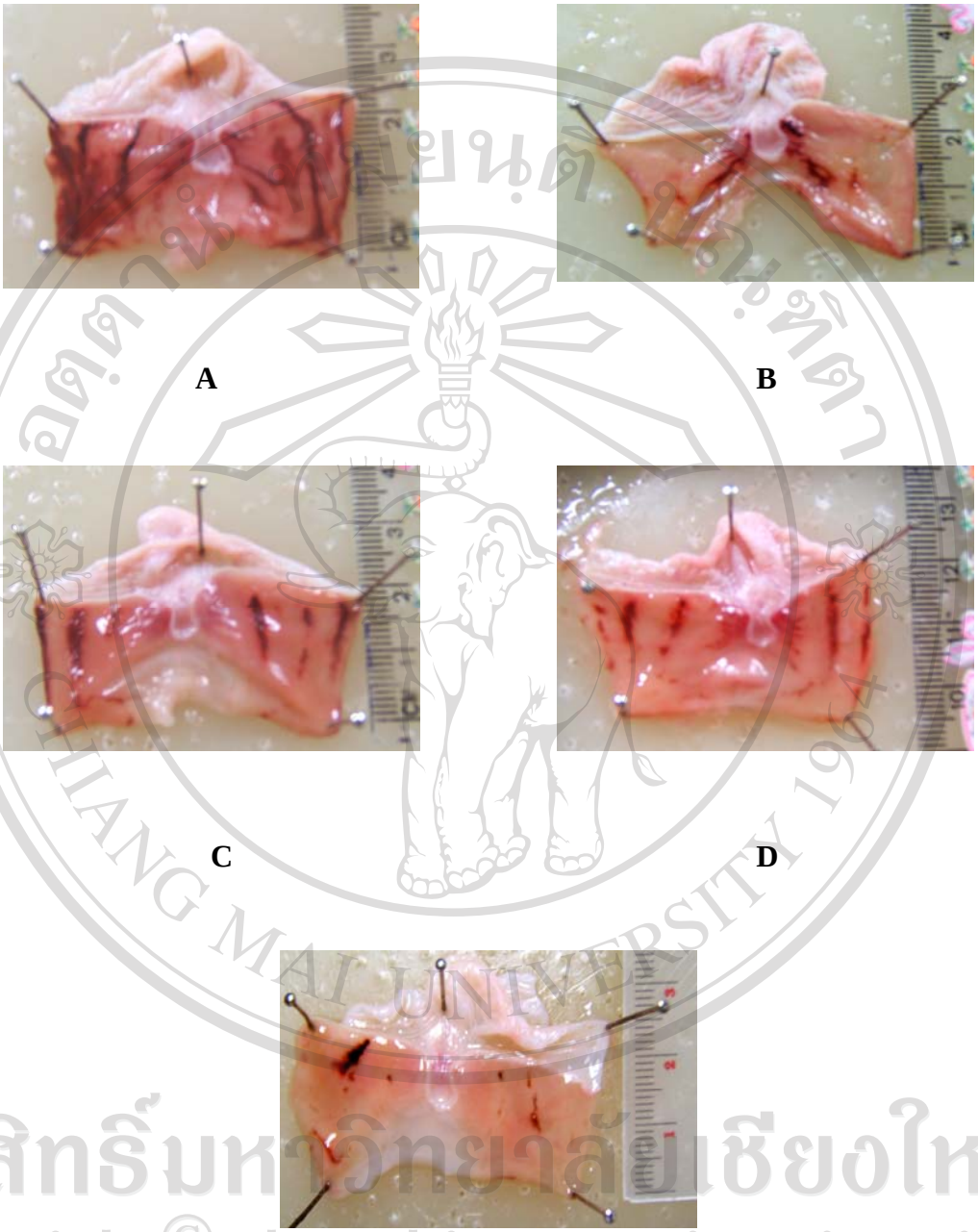

Figure 14 Gastric ulcers induced in rats by HCl/EtOH: A) control, B) cimetidine 100 mg/kg, C) *U. reticulata* 100 mg/kg, D) 200 mg/kg and E) 500 mg/kg

Table 2 Effect of the aqueous extract of *U. rcticulata* on HCl/Ethanol-induced gastric ulcers in rats

Group	Ulcer index (mm)	Inhibition (%)
Control	90.40 ± 10.06	-
Cimetidine		
100 mg/kg	29.50 ± 9.11 ***	67.37
U. reticulata		
100 mg/kg	72.68 ± 13.32	19.60
200 mg/kg	64.98 ± 10.56	28.12
500 mg/kg	6.05 ± 2.79 ***	93.31

Data expressed as mean ± S.E.M. (n = 8)

Significantly different from control group: (*** $p < 0.001$)

Indomethacin-induced gastric ulcers in rats

An intraperitoneal injection of indomethacin caused gastric ulceration in glandular mucosa of stomach which the most of lesions were small and petechiae lesions as shown in Figure 15. The aqueous extract of *U. rcticulata* at the doses of 100, 200 and 500 mg/kg exhibited anti-ulcer activity in indomethacin induced gastric ulcers in rats. Table 3 illustrates the effect of aqueous extract of *U. reticulata* and cimetidine on indomethacin-induced gastric ulcers in rats. Administration of indomethacin caused gastric ulceration with an ulcer index of 1.84 (control group). The aqueous extract of *U. reticulata* at the doses of 200 and 500 mg/kg significantly decreased ulcer formation with ulcer indexes of 0.19 and 0.16 mm and with percent inhibition of 89.67 and 91.30 respectively. Cimetidine also significantly decreased ulcer formation with ulcer index of 0.2 mm and percent inhibition of 89.13.

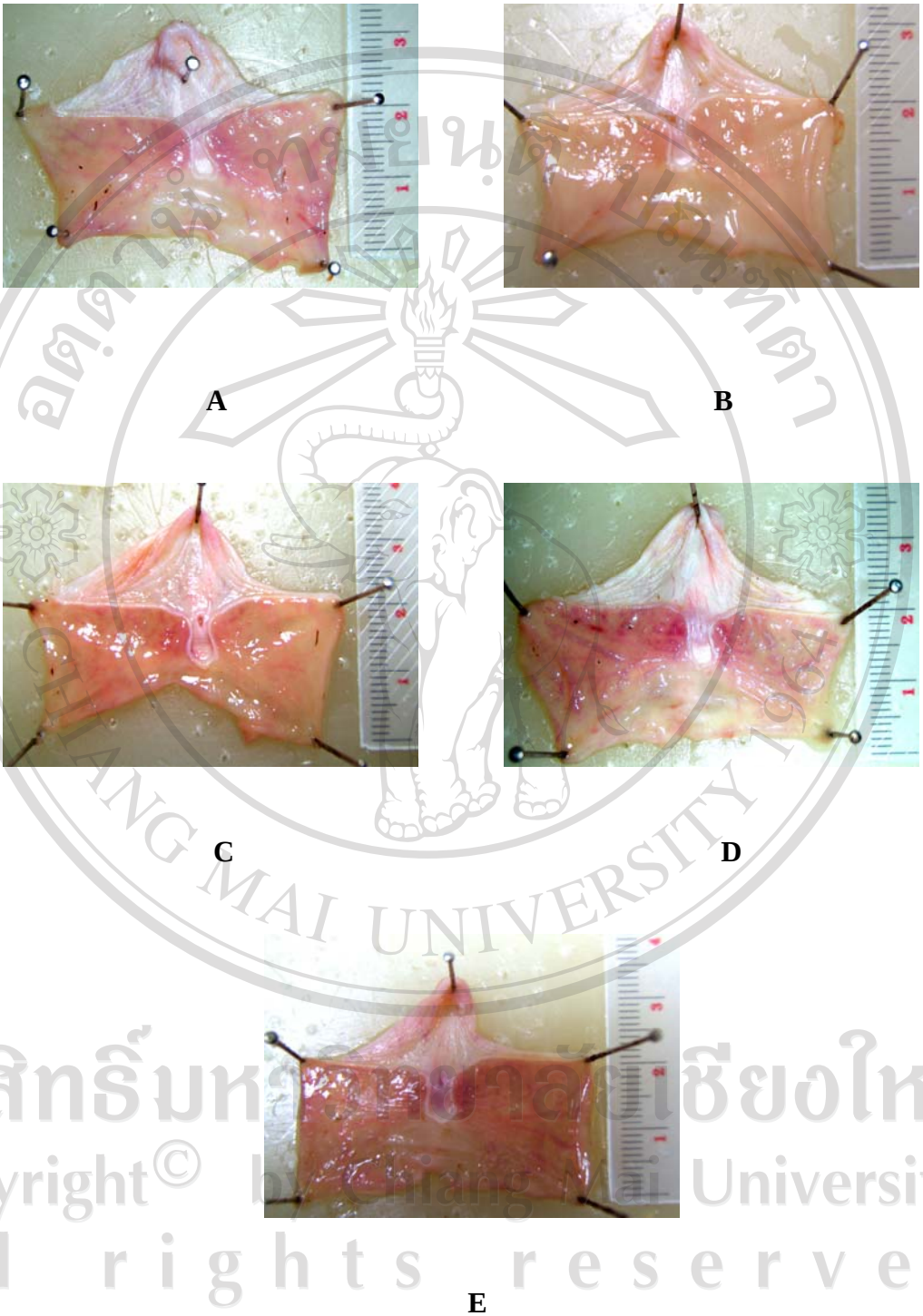

Figure 15 Gastric ulcers induced in rats by indomethacin: A) control, B) cimetidine 100 mg/kg, C) *U. reticulata* 100 mg/kg, D) 200 mg/kg and E) 500 mg/kg

Table 3 Effect of the aqueous extract of *U. reticulata* on indomethacin-induced gastric ulcers in rats

Group	Ulcer index (mm)	Inhibition (%)
Control	1.84 ± 0.67	-
Cimetidine		
100 mg/kg	0.20 ± 0.10 *	89.13
U. reticulata		
100 mg/kg	1.20 ± 0.78	34.78
200 mg/kg	0.19 ± 0.12 *	89.67
500 mg/kg	0.16 ± 0.10 *	91.30

Data expressed as mean ± S.E.M. (n = 8)

Significantly different from control group: (* $p < 0.05$)

Histamine-induced gastric ulcers in rats

An intraperitoneal injection of histamine caused gastric ulceration in the glandular segment of the stomach. The lesions consisted of small lesions**,** hemorrhagic spots were observed as depicted in Figure 16. Pretreatment with the aqueous extract of *U. reticulata* effectively reduced the gastric ulcer formation induced by histamine. The effects of the aqueous extract of *U. reticulata* and cimetidine on histamine-induced gastric ulcers are shown in Table 4. The aqueous extract of *U. reticulata* at the doses 100, 200 and 500 mg/kg significantly reduced ulcer formation and its effect was a dose-related manner. The doses of 100, 200 and 500 mg/kg showed ulcer indexes of 2.75, 0.95, 0.44 mm with percent inhibition of 60.43, 86.33, 93.67, respectively. The ulcer index of 0.66 mm and percent inhibition of 90.50 were observed with cimetidine administration.

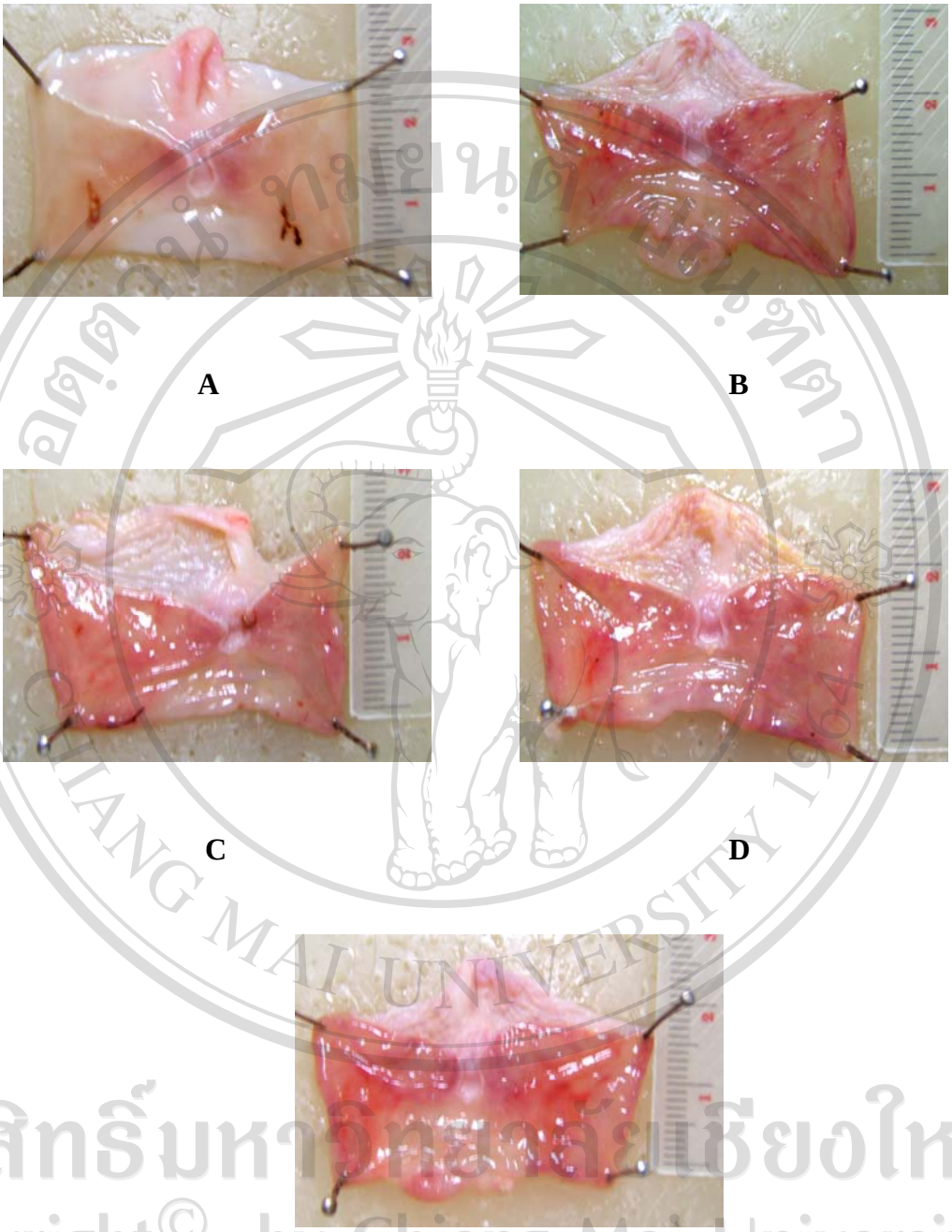

Figure 16 Gastric ulcers induced in rats by histamine: A) control, B) cimetidine 100 mg/kg, C) *U. reticulata* 100 mg/kg, D) 200 mg/kg and E) 500 mg/kg

Table 4 Effect of the aqueous extract of *U. reticulata* on histamine-induced gastric ulcers in rats

Group	Ulcer index (mm)	Inhibition (%)
Control	6.95 ± 2.23	-
Cimetidine		
100 mg/kg	0.66 ± 0.30 ***	90.50
U. reticulata		
100 mg/kg	2.75 ± 1.22 *	60.43
200 mg/kg	0.95 ± 0.30 ***	86.33
500 mg/kg	0.44 ± 0.37 ***	93.67

Data expressed as mean ± S.E.M. (n = 8)

Significantly different from control group: (* $p < 0.05$, *** $p < 0.001$)

Anti-gastric ulcer activity of the aqueous extract of *U. reticulata* in various gastric ulceration models

The anti-gastric ulcer activity (expressed as % inhibition of gastric ulcer formation) of the aqueous extract of *U. reticulata* when tested in 4 models of gastric ulceration including: restraint water immersion stress-, HCl/EtOH-, indomethacin- and histamine-induced gastric ulcers are shown in Figure 17. At the dose of 500 mg/kg, the aqueous extract of *U. reticulata* showed percent inhibition of gastric ulcer formation between 74-93 in all models. The aqueous extract of *U. reticulata* was less effective (% inhibition of 74.73) in the restraint water immersion stress model, whereas it was high effective in histamine-, HCl/EtOH- and indomethacin-induced gastric ulcers models with the percent inhibition of 93.67, 93.31 and 89.67, respectively.

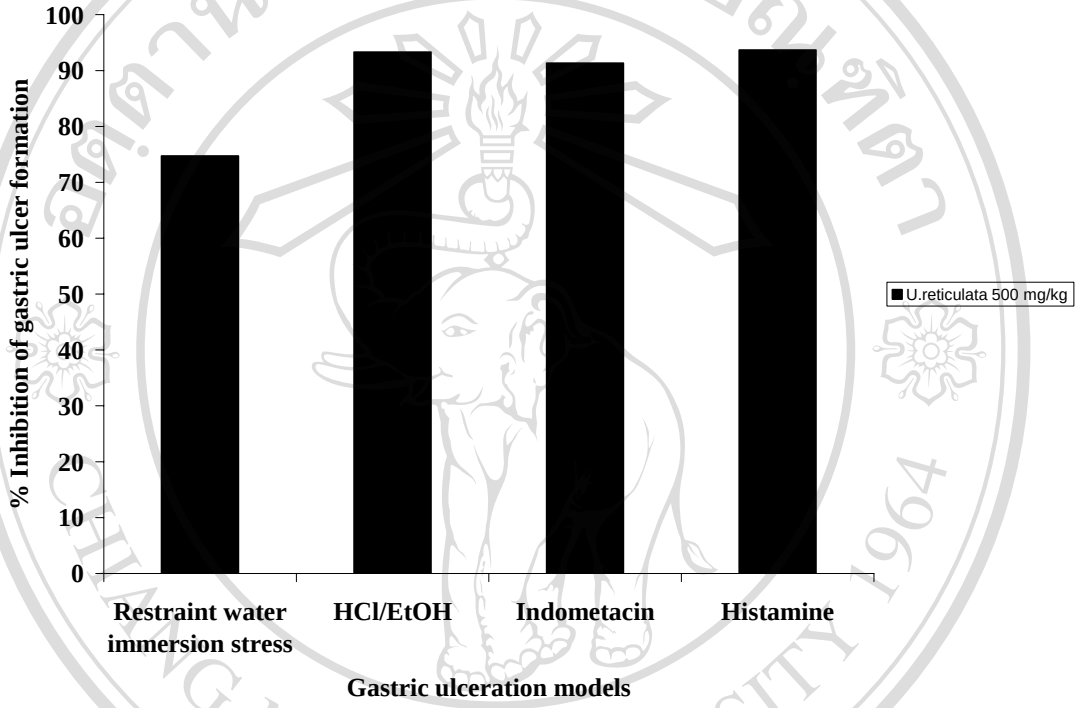

Figure 17 Anti-gastric ulcer activity (% inhibition of gastric ulcer formation) of the aqueous extract of *U. reticulata* at the dose of 500 mg/kg in different gastric ulceration models: 1) restraint water immersion stress, 2) HCl/EtOH, 3) indomethacin and 4) histamine-induced gastric ulcers in rats

Pyrolus ligation experiment

Table 5 illustrates the effect of the aqueous extract of *U. reticulata* on gastric volume, gastric pH and total acidity. The gastric volume of 2.87 ml/5h and gastric pH of 1.10 and total acidity 88.33 mEq/L were observed in the control group. Significant increased gastric pH to 2.17 and 2.21 were observed in groups treated with *U. reticulata* extract at the doses of 200 and 500 mg/kg, respectively. The aqueous extract of *U. reticulata* at the doses of 200 and 500 mg/kg caused significantly decrease of total acidity to 61.67 and 58.14 mEq/L, respectively. The gastric volume of groups receiving *U. reticulata* extract at the doses of 100, 200 and 500 mg/kg and were not statistically different from that of the control group. Cimetidine significantly decreased total acidity to 52.14 mEq/L and increased gastric pH to 7.39.

Table 5 Effect of the aqueous extract of *U. reticulata* on gastric secretion in pylorus ligated rats

Group	Gastric vol. (ml/5h)	Gastric pH	Acidity (mEq/L)
Control	2.87 ± 1.26	1.10 ± 0.36	88.33 ± 4.77
Cimetidine			
100 mg/kg	1.87 ± 0.78	7.39 ± 1.19***	52.14 ± 3.76**
U. reticulata			
100 mg/kg	2.23 ± 0.60	1.74 ± 0.55	73.33 ± 10.85
200 mg/kg	2.33 ± 0.85	2.17 ± 0.89*	61.67 ± 11.08*
500 mg/kg	3.62 ± 1.58	2.21 ± 1.10*	58.14 ± 4.76*

Data expressed as mean ± S.E.M. (n = 6)

Significantly different from control group: (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Gastric wall mucus with HCl/EtOH-induced gastric ulcers in rats

The effects of the aqueous extract of *U. reticulata* and cimetidine on gastric wall mucus of rats with HCl/EtOH-induced gastric ulcer are shown in Table 6. In the normal group (without HCl/EtOH-induced gastric ulcer) had gastric wall mucus of 280.73 ug alcian blue/g wet stomach. The treatment with HCl/EtOH resulted in a decrease of gastric wall mucus. As seen in the control group (which received vehicle), the gastric wall mucus was significantly less than that of the normal group. Pretreatment with the aqueous extract of *U. reticulata* at doses of 100, 200 and 500 mg/kg did not protect the loss of gastric wall mucus caused by HCl/EtOH administration. The gastric wall mucus contents of groups treated with 100, 200 and 500 mg/kg of *U. reticulata* extract were found to be 141.71, 154.76 and 166.87 ug alcian blue/g wet stomach, respectively which were not significantly different from that of HCl/EtOH treated (control) group. The gastric wall mucus of the cimetidine group was 200.33 ug alcian blue/g wet stomach which was not significantly different from that of the control group.

Table 6 Effect of the aqueous extract of *U. reticulata* on gastric wall mucus content of rats with HCl/EtOH-induced gastric ulcers

Group	Gastric wall mucus (ug Alcian blue/g wet stomach)
Normal	280.73 ± 49.09
HCl/EtOH treated rats	
Control	138.66 ± 4.90*
Cimetidine	
100 mg/kg	200.33 ± 12.08
U. reticulata	
100 mg/kg	141.71 ± 10.21
200 mg/kg	154.76 ± 15.15
500 mg/kg	166.87 ± 32.49

Data expressed as mean ± S.E.M. (n = 6)

Significantly different from normal group: (* $p < 0.001$)

Isolated guinea-pig right atria experiment

Histamine induced increased heart rate when tested on the isolated guinea-pig right atria. The administration of cimetidine (Figure 18) or the aqueous extract of *U. reticulata* (Figure 19) during the sustained increased heart rate induced by histamine resulted in decrease of the heart rate. Increasing concentration of cimetidine or the aqueous extract of *U. reticulata* produced increasing responses. Table 7 illustrates the inhibitory effect of the aqueous extract of *U. reticulata* and cimetidine on histamine induced increased heart rate. The aqueous extract of *U. reticulata* at the doses of 0.5, 1 and 2 mg/ml caused % inhibition of histamine induced increased heart rate of 26.05, 38.59 and 59.45, respectively. Cimetidine at the doses of 0.25, 0.5 and 1 ug/ml could inhibit histamine induced increased heart rate to 30.48, 51.56 and 68.59 %, respectively. The effects of the aqueous extract of *U. reticulata* and cimetidine were concentration dependent, with r values of 0.98 and 0.99, respectively. The EC50 of the aqueous extract of *U. reticulata* and cimetidine were 1.43 and 0.5, respectively. Figure 20 depicts the regression lines of concentration-response (inhibition of histamine induced increased heart rate) of cimetidine and the aqueous extract of *U. reticulata*. The slope values of the regression lines of the aqueous extract of *U. reticulata* and cimetidine were found to be 55.46 and 63.29 which were not statistically different, thus suggesting that they are parallel.

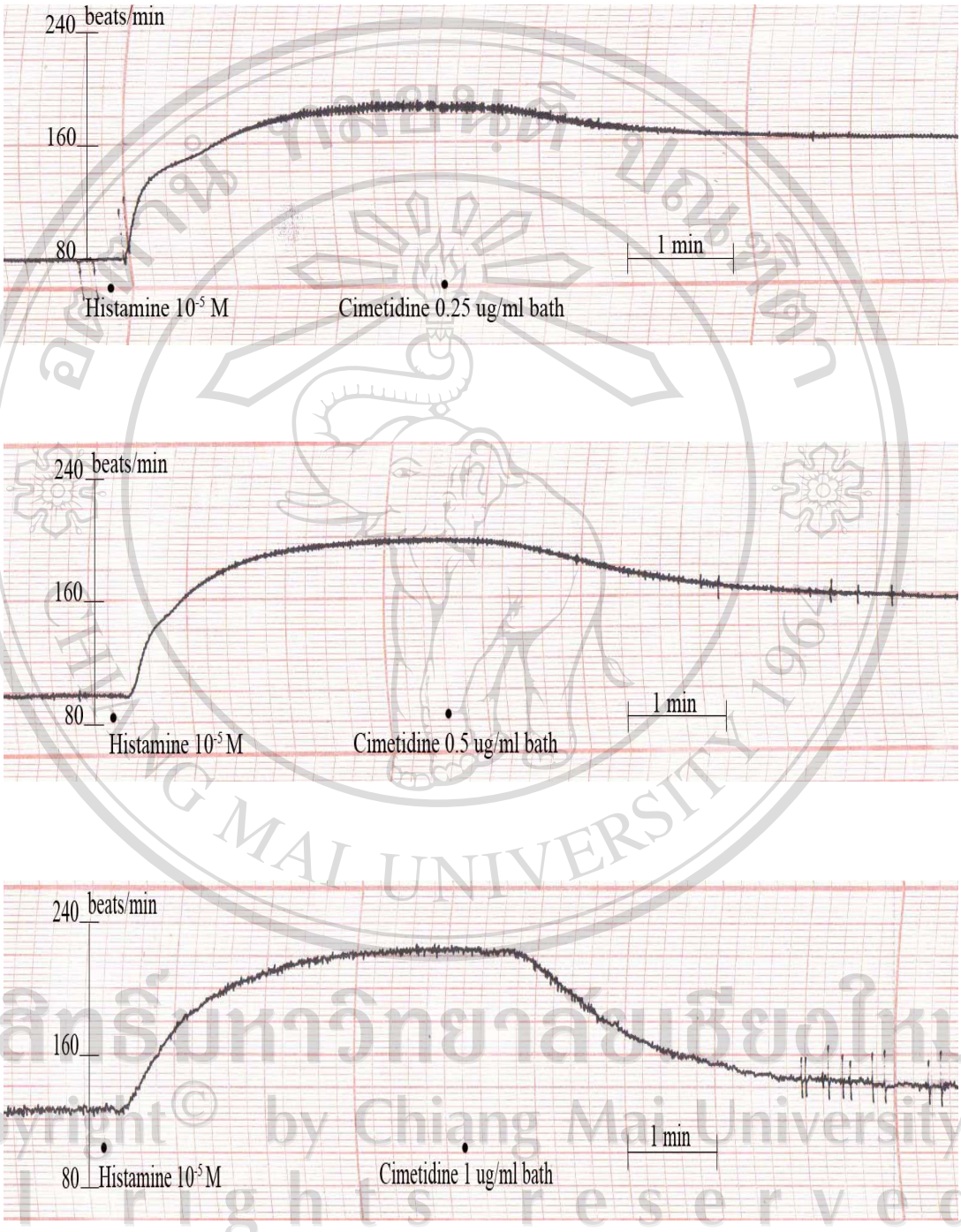

Figure 18 Effect of cimetidine on histamine induced increased heart rate of isolated guinea-pig right atria

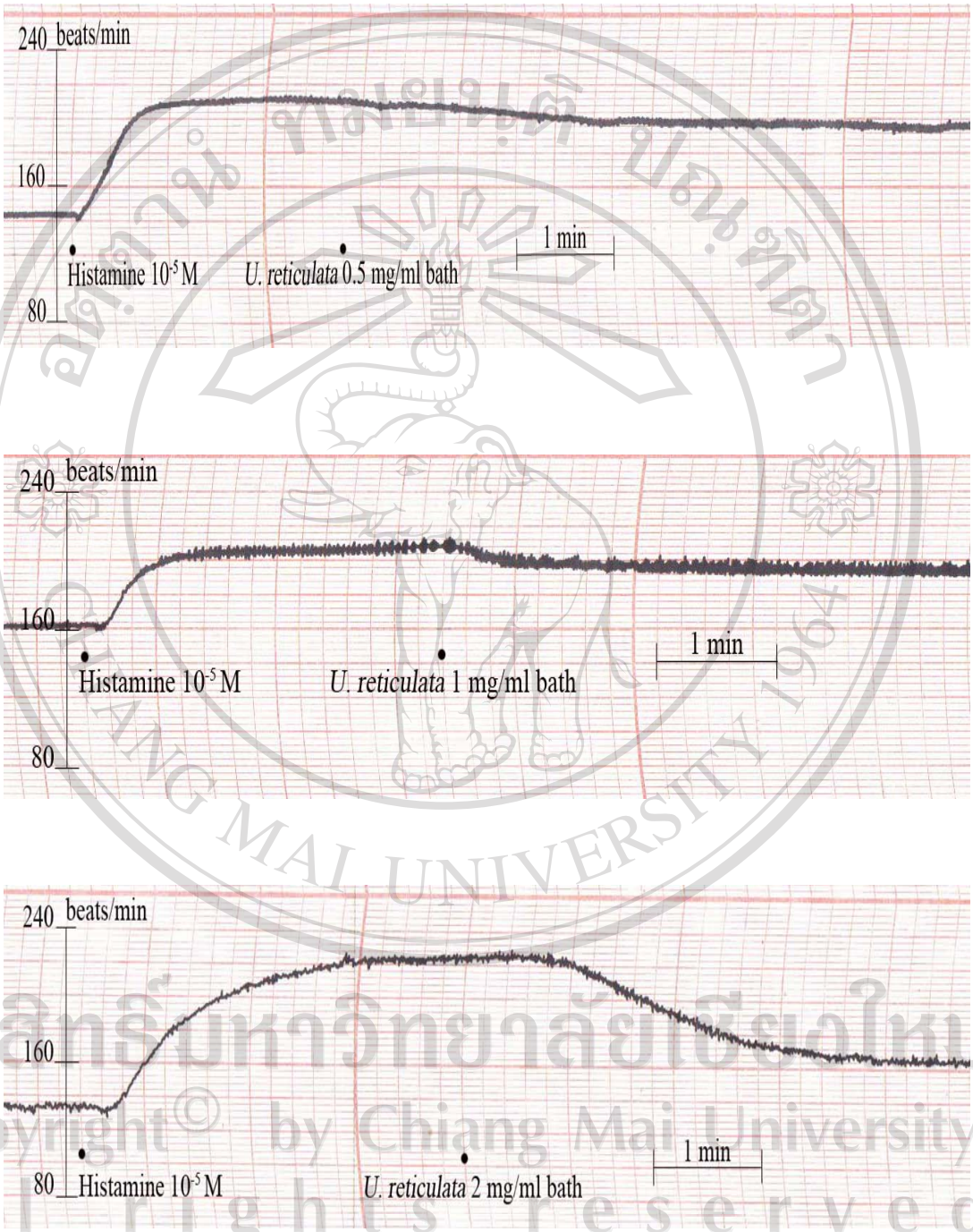

Figure 19 Effect of the aqueous extract of *U. reticulata* on histamine induced increased heart rate of isolated guinea-pig right atria

Table 7 The inhibitory effect of cimetidine and the aqueous extract of *U. reticulata* on histamine induced increased heart rate of the isolated right guinea-pig atria

Group	% Inhibition of histamine induced increased heart rate
Cimetidine	
0.25 ug/ml	30.48 ± 4.42
0.5 ug/ml	51.46 ± 5.14
1 ug/ml	68.59 ± 7.15
U. reticulata	
0.5 mg/ml	26.05 ± 2.50
1 mg/ml	39.59 ± 6.85
2 mg/ml	59.45 ± 11.69

Data expressed as mean ± S.E.M. (n = 6)

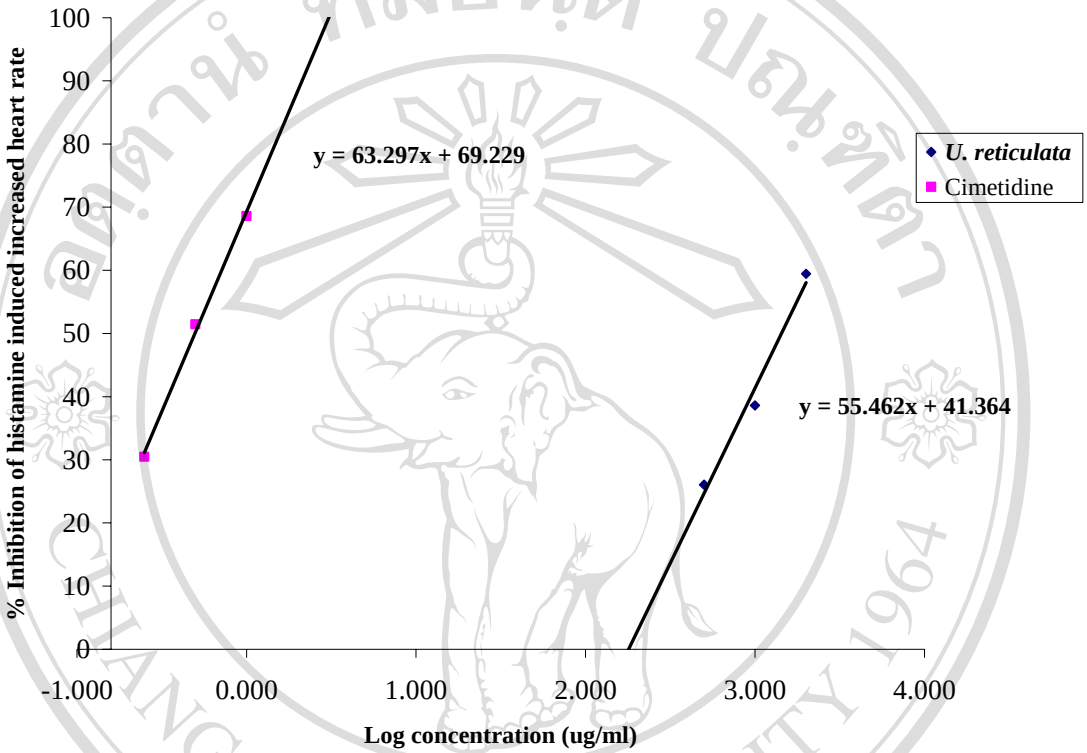

Figure 20 Concentration-response (the inhibitory effect of the aqueous extract of *U. reticulata* and cimetidine on histamine induced increased heart rate of the isolated guinea-pig right atria) regression lines of the aqueous extract of *U. reticulata* and cimetidine