CHAPTER 3

RESULTS

3.1 Phytochemical screening test

The results showed that TIE extract presented primary and/or secondary and/or tertiary alkaloids and coumarins.

3.2 Analgesic study

3.2.1 Writhing response

The inhibitory effects of TI extract and diclofenac on acetic acid-induced writhing response in mice are demonstrated in Figure 3.1 and Table 3.1. In the test group, all doses of TI extract showed a significant inhibitory effect on acetic acid-induced writhing response in a dose-dependent manner (R^2 = 0.9953). Similarly, diclofenac at the dose of 10 mg/kg showed a marked inhibition on writhing response.

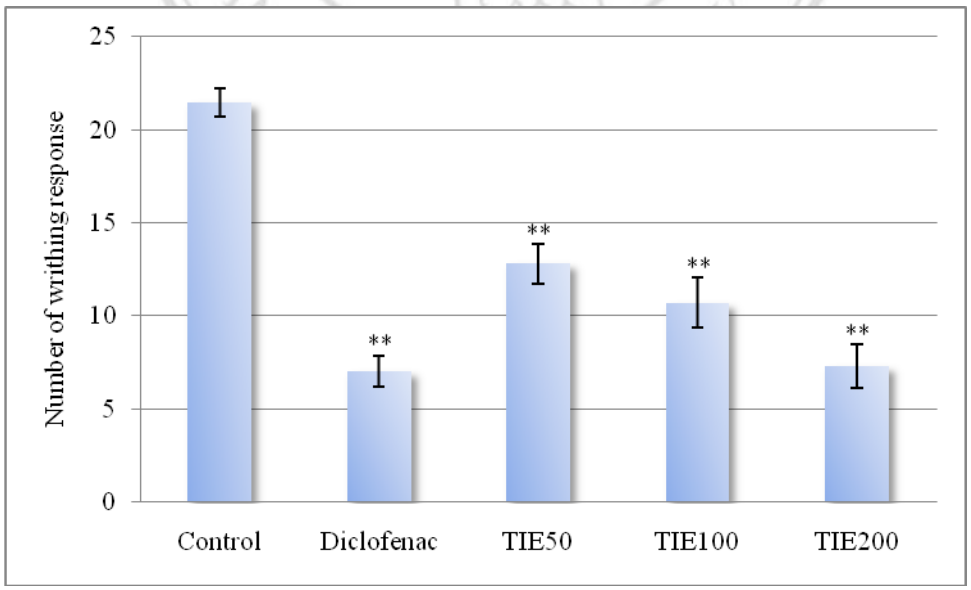

Figure 3.1 Effects of TI extract and diclofenac on acetic acid-induced writhing response in mice. Significant difference from control (5% Tween80); ** = P<0.001.

Table 3.1 Effects of TI extract and diclofenac on acetic acid-induced writhing response in mice.

Group	**Dose** (mg/kg)	**nWR**	**%WI**
Control	-	21.5 ± 0.76	-
Diclofenac	10	7.0 ± 0.82 **	67
TI extract	50	12.8 ± 1.08 **	40
TI extract	100	10.7 ± 1.33 **	50
TI extract	200	7.3 ± 1.17 **	66

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

nWR = number of writhing response

%WI = percent writhing response inhtbition of test substance at time

Significant difference from control (5% Tween80); ** $P<0.001$.

3.2.2 Tail-flick test

The inhibitory effects of TI extract, diclofenac and codeine on tail-flick test in rats are shown in Figure 3.2 and Table 3.2. The reaction time of the groups which received either the maximal dose used in this study of TI extract (200 mg/kg) or diclofenac did not differ from that of the control group. In contrast, the reaction time of codeine-treated group was significantly increased, thus codeine exhibited a marked inhibitory effect on the tail-flick response rats.

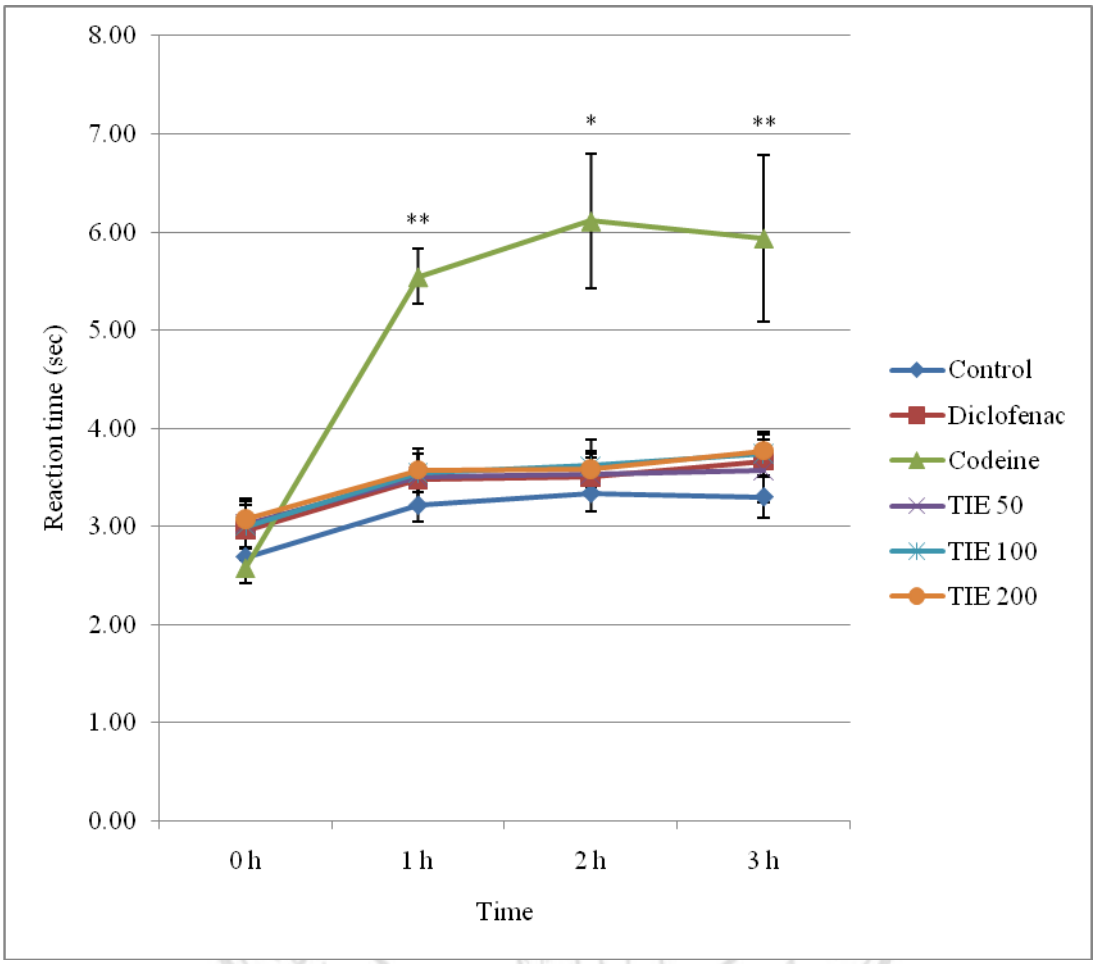

Figure 3.2 Effects of TI extract, diclofenac and codeine on the tail-flick test in rats. Significant difference from control (5% Tween80); * = $P<0.05$, ** = $P<0.001$.

Table 3.2 Effects of TI extract, diclofenac and codeine on the tail-flick test in rats.

Group	Dose (mg/kg)	T_b (sec)	T_t (sec)			Maximum possible response (%)		
			1 h	2 h	3 h	1 h	2 h	3 h
Control	-	2.69 ± 0.27	3.22 ± 0.17	3.34 ± 0.18	3.30 ± 0.21	-	-	-
Diclofenac	10	2.96 ± 0.26	3.48 ± 0.26	3.51 ± 0.20	3.67 ± 0.16	7	7	9
Codeine	200	2.58 ± 0.08	5.55 ± 0.28 **	6.11 ± 0.69 *	5.94 ± 0.85 **	40	48	45
TI extract	50	3.03 ± 0.25	3.51 ± 0.29	3.53 ± 0.21	3.57 ± 0.32	7	7	8
TI extract	100	3.00 ± 0.21	3.55 ± 0.20	3.62 ± 0.26	3.74 ± 0.22	8	9	10
TI extract	200	3.07 ± 0.19	3.58 ± 0.17	3.59 ± 0.18	3.77 ± 0.17	7	7	10

Data are expressed as mean ± S.E.M. (n=6). Control received 5% Tween80.

T_b = baseline reaction time

T_t = reaction time after receiving of test drug

Significant difference from control (5% Tween80); * $P<0.05$, ** $P<0.001$.

3.3 Anti-inflammatory study

3.3.1 EPP-induced ear edema in rats

The ear thickness of rats in the control group (received acetone only) increased gradually and the maximum edema was observed at 120 min after EPP application. Diclofenac and TI extract at the dose of 3 mg/ear significantly reduced the edema formation at all assessment times (Figure 3.3). The inhibitory effects produced by topical administration of TI extract and diclofenac on EPP-induced ear edema are shown in Table 3.3.

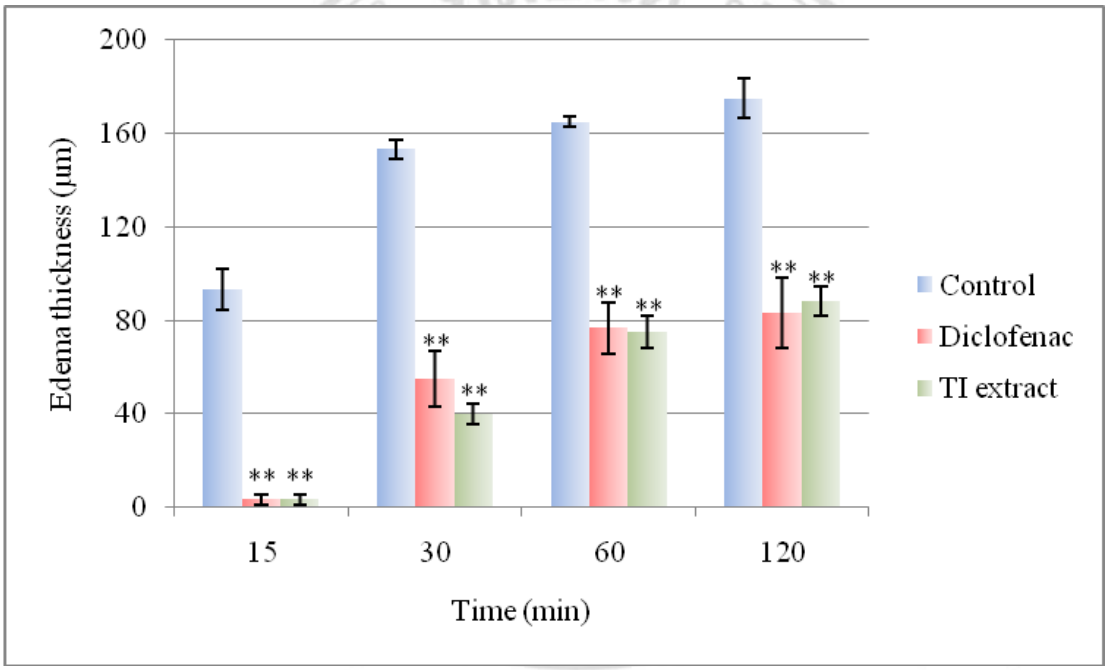

Figure 3.3 Effects of TI extract and diclofenac on EPP-induced ear edema in rats. Significant difference from control (acetone); ** = $P<0.001$.

Table 3.3 Effects of TI extract and diclofenac on EPP-induced ear edema in rats.

Group	Dose (mg/ear)	Edema thickness (μm)				% Edema inhibition			
		15 min	30 min	60 min	120 min	15 min	30 min	60 min	120 min
Control	-	93.3 ± 8.8	153.3 ± 4.2	165.0 ± 2.2	175.0 ± 8.5	-	-	-	-
Diclofenac	3	3.3 ± 2.1**	55.0 ± 11.8**	76.7 ± 10.9**	83.3 ± 14.8**	96	64	54	52
TI extract	3	3.3 ± 2.1**	40.0 ± 4.5**	75.0 ± 6.7**	88.3 ± 6.0**	96	74	55	50

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

Significant difference from control (acetone); ** $P<0.001$.

3.2.2 Carrageenan-induced hind paw edema in rats

In the control group (5% Tween80), the edema volume of rat paw was found to increase gradually and reached the maximal response at the 3rd h after carrageenan injection. Diclofenac at the dose of 10 mg/kg body weight exhibited a significant edema inhibition at all recorded time. Likewise TI extract at the doses of 50, 100 and 200 mg/kg body weight showed a dose-dependent edema inhibition at all recorded times (R^2 = 0.9440, 0.9826, 0.8342 at the 1st, 3rd and 5th h, respectively) except that of the dose of 50 mg/kg body weight at the 5th h (Figure 3.4). The inhibitory activities of TI extract and diclofenac on carrageenan-induced hind paw edema in rats are presented in Table 3.4.

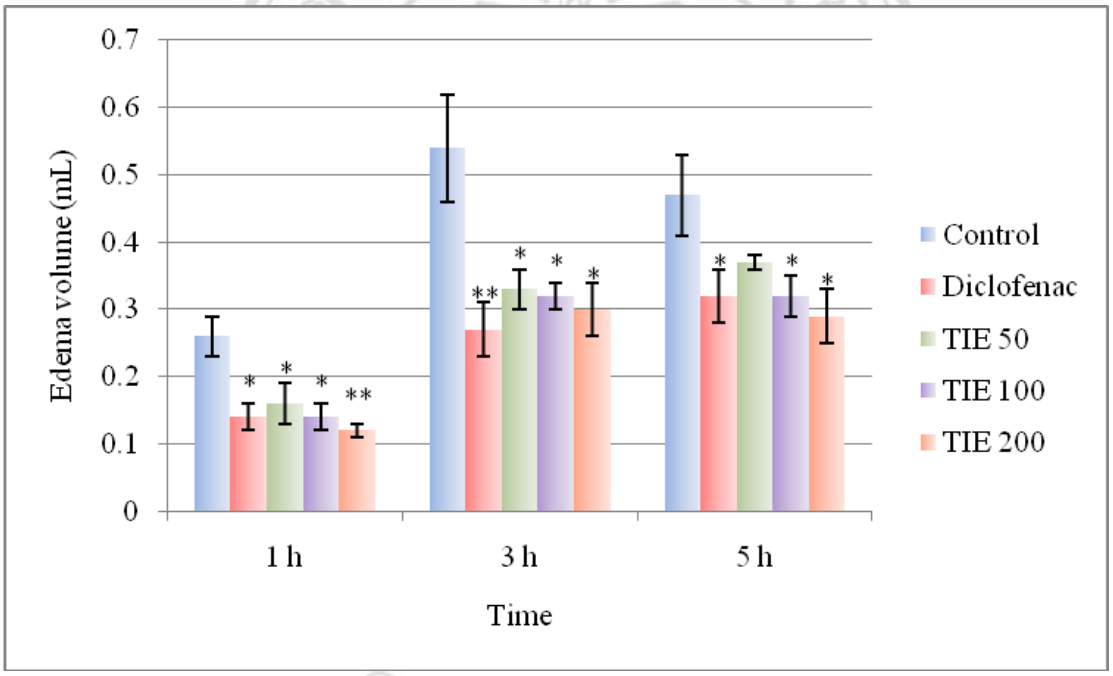

Figure 3.4 Effects of TI extract and diclofenac on Carrageenan-induced hind paw edema in rats. Significant difference from control (5% Tween80); * = P<0.05, ** = P<0.001.

Table 3.4 Effects of TI extract and diclofenac on Carrageenan-induced hind paw edema in rats.

Group	**Dose**	**Edema volume** (mL)			**% Edema inhibition**		
	(mg/kg)	**1 h**	**3 h**	**5 h**	**1 h**	**3 h**	**5 h**
Control	-	0.26 ± 0.03	0.54 ± 0.08	0.47 ± 0.06	-	-	-
Diclofenac	10	0.14 ± 0.02*	0.27 ± 0.04**	0.32 ± 0.04*	46	50	33
TI extract	50	0.16 ± 0.03*	0.33 ± 0.03*	0.37 ± 0.01	39	38	20
TI extract	100	0.14 ± 0.02*	0.32 ± 0.02*	0.32 ± 0.03*	48	41	32
TI extract	200	0.12 ± 0.01**	0.30 ± 0.04*	0.29 ± 0.04*	55	45	37

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

Significant difference from control (5% Tween80); * $P<0.05$, ** $P<0.001$.

3.2.3 Arachidonic acid (AA)-induced hind paw edema in rats

In the control group, the injection of 0.5% AA into the plantar side of the right hind paw significantly produced edema formation by 1 h after challenge. Diclofenac and TI extract at the dose of 50 mg/kg did not show significant inhibitory effect on the edema formation of the rat paw induced by AA. In contrast, prednisolone at the dose of 5 mg/kg body weight, TE extract at the dose of 100 and 200 mg/kg body weight exhibited a significant inhibitory activity and a dose-dependent manner (R^2 = 0.8242) on the edema formation (Figure 3.5, Table 3.5).

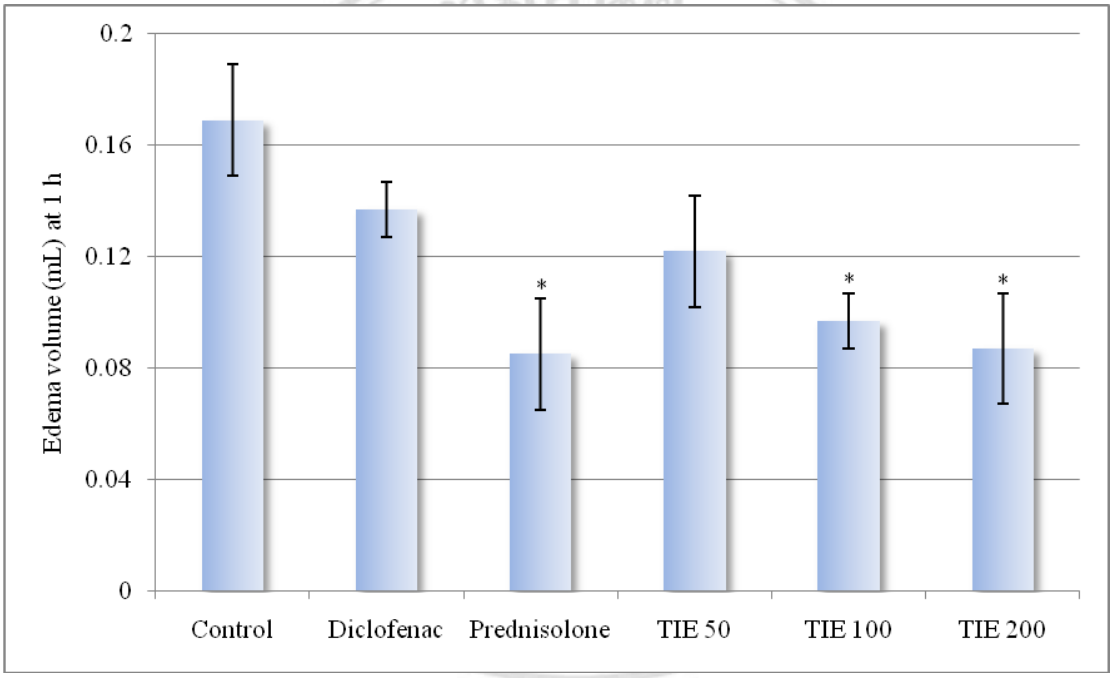

Figure 3.5 Effects of TI extract, diclofenac and prednisolone on AA-induced hind paw edema in rats. Significant difference from control (5% Tween80); * = P<0.05.

Table 3.5 Effects of TI extract, diclofenac and prednisolone on AA-induced hind paw edema in rats.

Treatment	**Dose** (mg/kg)	**Edema volume** (mL) **at 1 h**	**% Edema inhibition**
Control	-	0.169 ± 0.02	-
Diclofenac	10	0.137 ± 0.01	19
Prednisolone	5	0.085 ± 0.02 *	50
TI extract	50	0.122 ± 0.02	28
TI extract	100	0.097 ± 0.01 *	43
TI extract	200	0.087 ± 0.02 *	49

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

Significant difference from control (5% Tween80); * $P<0.05$

3.2.4 Cotton pellet-induced granuloma formation in rats

Granuloma formation

The summarized effect of TI extract, diclofenac and prednisolone on cotton pellet-induced granuloma formation in rats is shown in Figure 3.6. Prednisolone significantly reduced the transudative weight and inhibited granuloma formation, and diclofenac significantly inhibited only granuloma formation. However, TI extract did not produce any inhibitory effect on both transudative weight and granuloma formation (Table 3.6).

Body weight and thymus weight

Body weight gain and thymus weight of rats in TI extract- and diclofenac-treated groups were not significantly different from those of the control group. On the contrary, both parameters of the prednisolone-treated animals were reduced markedly when compared with those of the control group (Table 3.7).

Alkaline phosphatase activity

The serum ALP activity in the control group (33.27 ± 3.50 unit x 10^{-4}/mg) was significantly increased when compared with that of the normal non-implanted group (23.87 ± 0.61 unit x 10^{-4}/mg). After 7 days of treatment with diclofenac or prednisolone, the serum ALP activity of both groups was significantly decreased when compared with

that of the control group. In TI extract-treated group, the serum ALP activity was not different from that of the control group (Table 3.8).

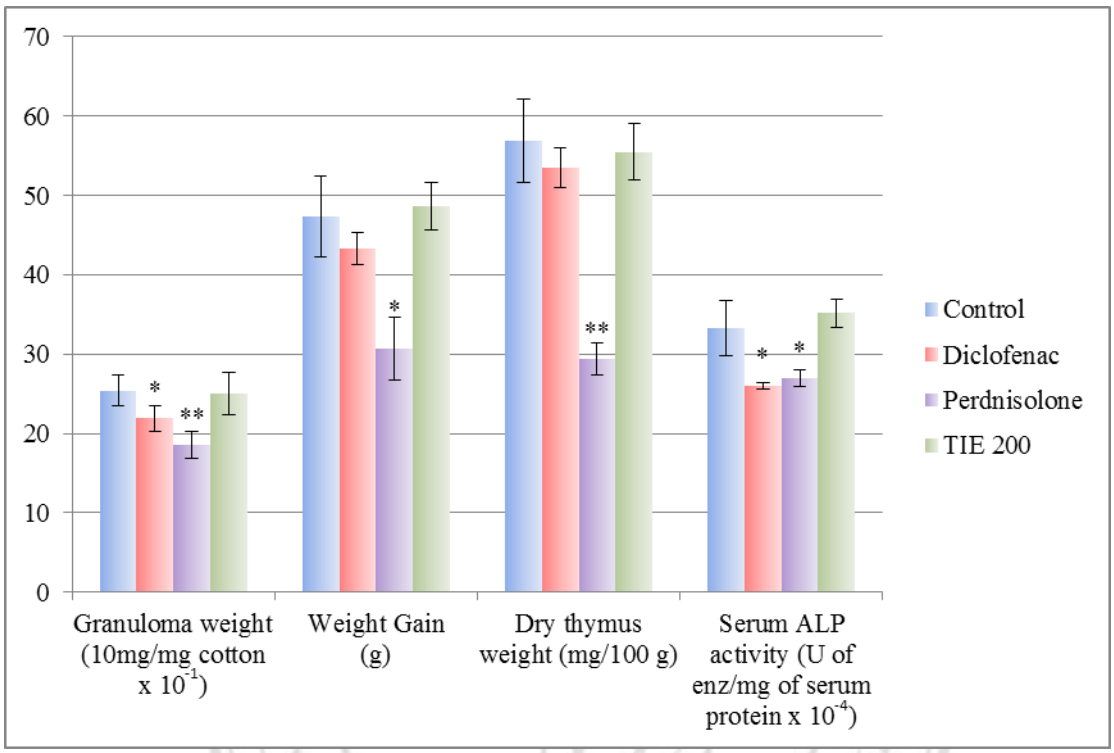

Figure 3.6 Effects of TI extract, diclofenac and prednisolone on granuloma weight, weight gain, thymus weight and serum ALP activity of rats in cotton pellet-induced granuloma formation model. Significant difference from control (5% Tween80); * = $P<0.05$, ** $P<0.001$.

Gastric ulcer

There was no gastric ulcer formation in all treated groups of rats after oral administration of each substance for 7 days (Figure 3.7).

Table 3.6 Effects of TI extract, diclofenac and prednisolone on granuloma formation and transudative weight in cotton pellet-induced granuloma formation in rats.

Group	**Dose** (mg/kg)	**Granuloma wet weight** (mg)	**Granuloma dry weight** (mg)	**Transudative weight** (mg)	**Granuloma weight** (mg/mg cotton)	**Granuloma inhibition** (%)
Control	-	260.16 ± 12.97	50.73 ± 1.88	209.43 ± 11.43	2.54 ± 0.09	-
Diclofenac	5	229.81 ± 12.91	43.88 ± 1.20 *	185.93 ± 12.40	2.19 ± 0.06 *	14
Prednisolone	5	175.43 ± 6.22 **	37.06 ± 1.48 **	138.38 ± 5.06 **	1.85 ± 0.07 **	27
TI extract	200	275.42 ± 13.78	50.12 ± 3.42	225.30 ± 12.95	2.50 ± 0.17	1

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

Significant difference from control (5% Tween80); * $P<0.05$, ** $P<0.001$.

Table 3.7 Effects of TI extract, diclofenac and prednisolone on body weight and dry thymus weight in cotton pellet-induced granuloma formation in rats.

Group	Dose	Body weight (g)			Dry thymus weight
	(mg/kg)	Initial	Final	Gain	(mg/100 g)
Control	-	173.3 ± 8.0	220.7 ± 7.1	47.3 ± 5.1	56.9 ± 5.2
Diclofenac	5	189.0 ± 3.9	232.3 ± 4.4	43.3 ± 2.0	53.5 ± 2.5
Perdnisolone	5	167.0 ± 6.8	197.7 ± 6.6	30.7 ± 3.9 *	29.4 ± 2.1 **
TI extract	200	182.0 ± 5.6	230.7 ± 6.5	48.7 ± 3.0	55.5 ± 3.5

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

Significant difference from control (5% Tween80); * $P<0.05$, ** $P<0.001$.

Table 3.8 Effects of TI extract, diclofenac and prednisolone on serum ALP activity in cotton pellet-induced granuloma formation in rats.

Group	Dose (mg/kg)	Alkaline phosphatase (U/L)	Total protein (g/dL)	Serum ALP activity (U of enz/mg of serum protein x 10^{-4})
Normal non-implanted	-	128.1 ± 3.8	5.30 ± 0.03	23.87 ± 0.61 *
Control	-	169.0 ± 20.0	5.05 ± 0.06	33.27 ± 3.50
Diclofenac	5	126.3 ± 3.1	4.85 ± 0.07	26.04 ± 0.41 *
Prednisolone	5	143.8 ± 6.4	5.33 ± 0.07	26.95 ± 1.07 *
TI extract	200	182.0 ± 8.3	5.18 ± 0.06	35.15 ± 1.74

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

Significant difference from control (5% Tween80); * $P<0.05$.

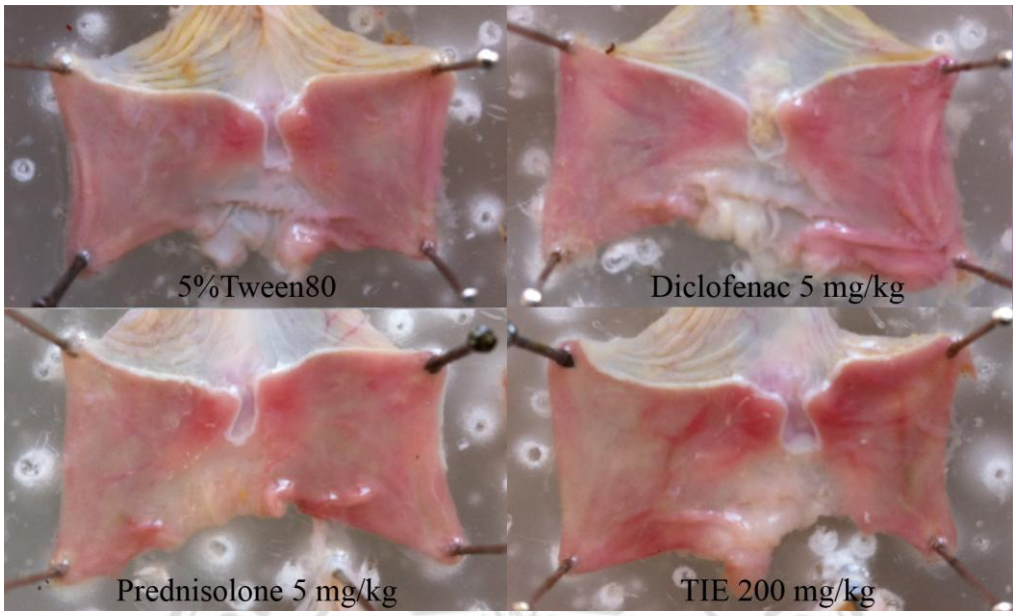

Figure 3.7 Illustrations of the stomachs after drug administration for 7 days on cotton pellet-induced granuloma formation in rats.

3.3 Anti-ulcerogenic study

3.3.1 Gastric ulcer prevention

3.3.1.1 Ethanol/hydrochloric acid (EtOH/HCl)-induced gastric lesions

An oral administration of EtOH/HCl to 48 h fasted rats resulted in severe gastric mucosa damage. In the control group, hemorrhagic elongated bands in the glandular segment of the stomach were clearly observed (Figure 3.8). TI extract at the doses of 50, 100, and 200 mg/kg, and ranitidine at the dose of 100 mg/kg significantly prevented gastric ulcer formation when compared with that of the control group (Figure 3.9, Table 3.9). TI extract at the highest dose used in this study exerted more effective in decreasing the formation of gastric ulcer than that of ranitidine. This inhibitory effect of TI extract seems to be in a dose-dependent manner.

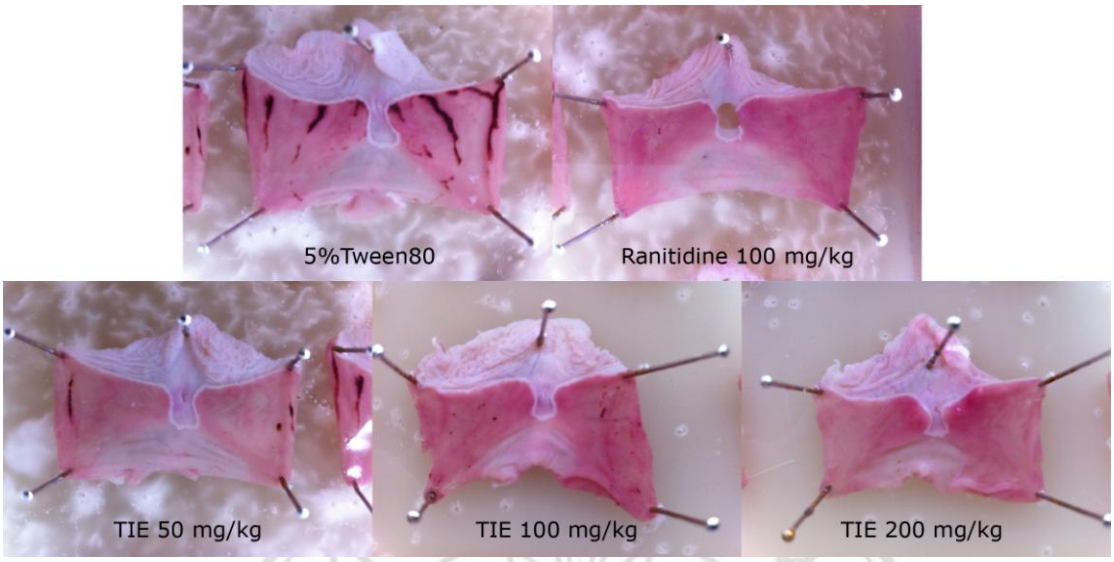

Figure 3.8 Illustrations of gastric ulcer prevention after pretreatment with TI extract and ranitidine compared with the control group on EtOH/HCl-induced gastric ulcer in rats.

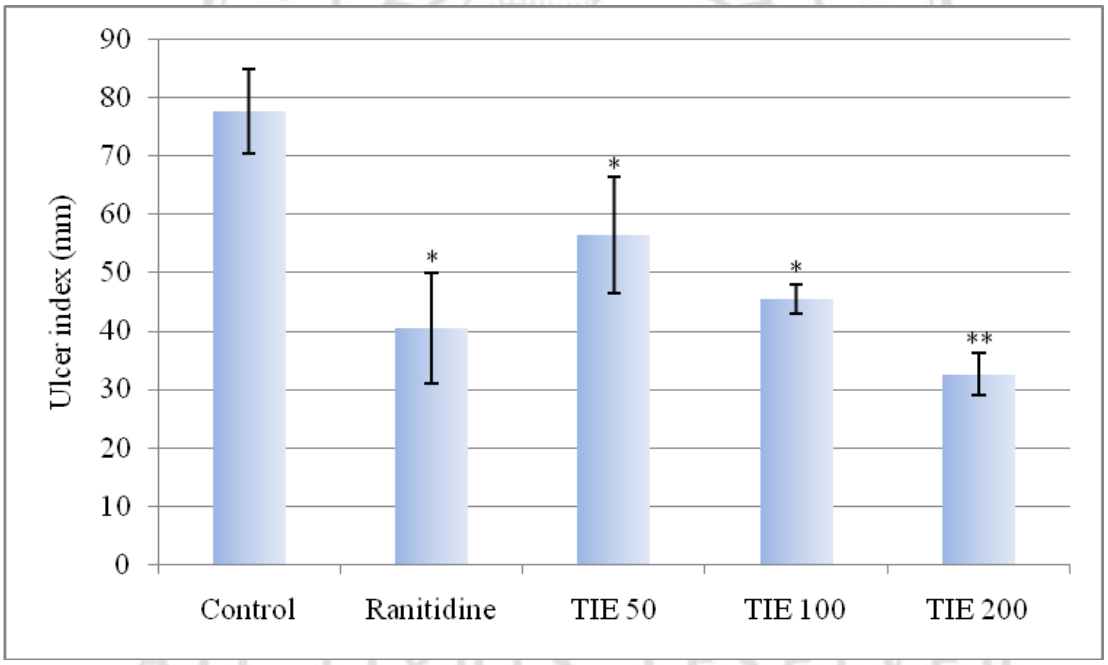

Figure 3.9 Effects of TI extract and ranitidine on EtOH/HCl-induced gastric lesions. Significant difference from control (5% Tween80); * $P<0.05$, ** $P<0.001$.

Table 3.9 Effects of TI extract and ranitidine on EtOH/HCl-induced gastric lesions.

Group	Dose (mg/kg)	Ulcer index (mm)	Inhibition (%)
Control	-	77.75 ± 7.27	-
Ranitidine	100	40.50 ± 9.39 *	48
TI extract	50	56.50 ± 9.98 *	27
TI extract	100	45.50 ± 2.62 *	41
TI extract	200	32.58 ± 3.66 **	58

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

Significant difference from control (5% Tween80); * $P<0.05$, ** $P<0.001$.

3.3.1.2 Indomethacin-induced gastric lesions

An oral administration of indomethacin caused gastric lesion in glandular mucosa of the stomach which the most of lesions were small and petechiae as shown in Figure 3.10. A statistically significant decrease of ulcer indices was found in the TI extract-treated groups at the doses of 100 and 200 mg/kg and ranitidine-treated group when compared with that of the control group (Figure 3.11, Table 3.10).

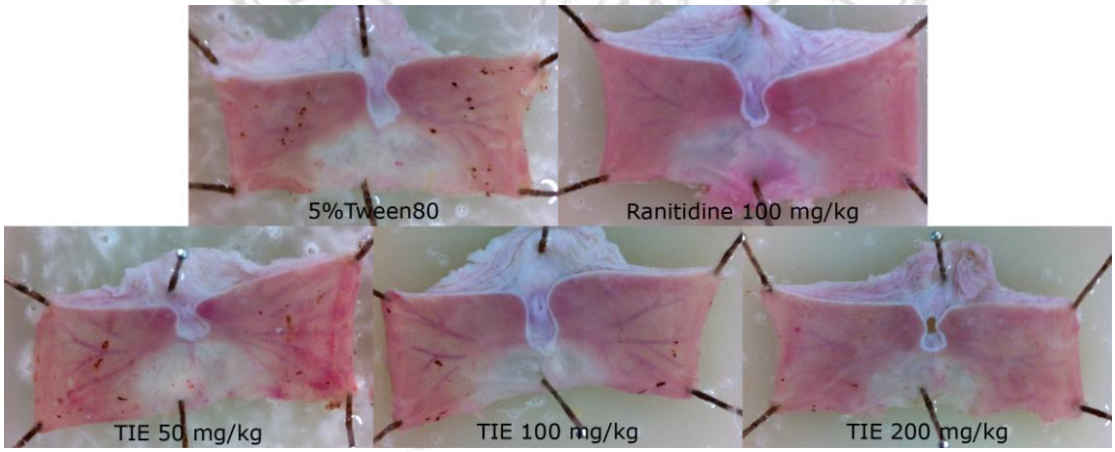

Figure 3.10 Illustrations of gastric ulcer after pretreatment with TI extract and ranitidine compared with the control group on indomethacin-induced gastric ulcer in rats.

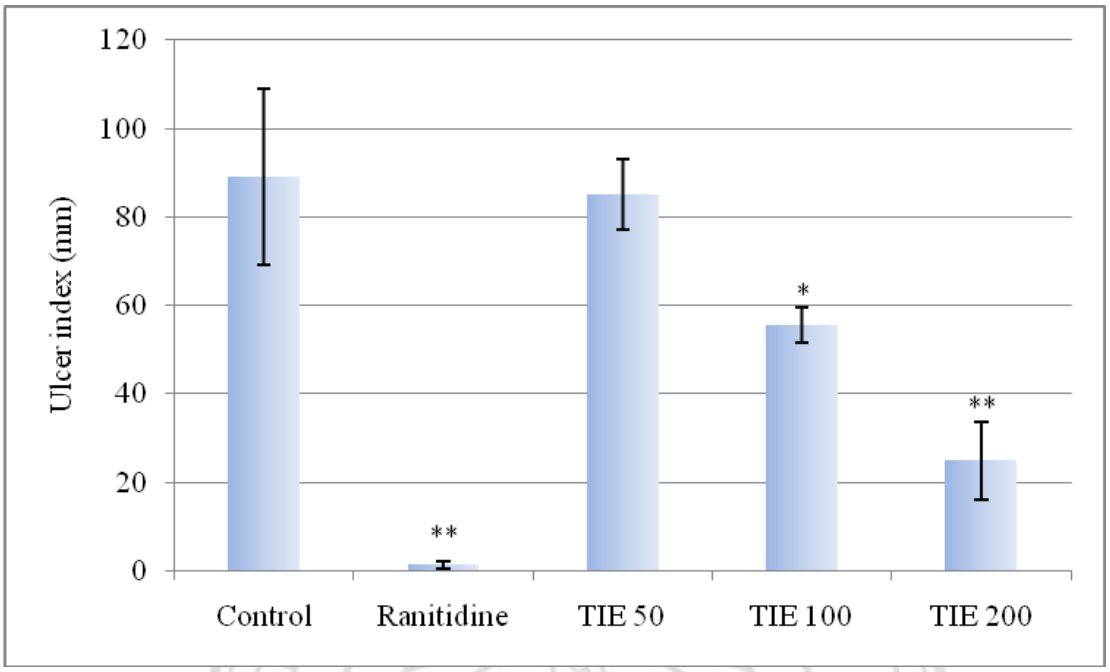

Figure 3.11 Effects of TI extract and ranitidine on indomethacin-induced gastric lesions. Significant difference from control (5% Tween80); * $P<0.05$, ** $P<0.001$.

Table 3.10 Effects of TI extract and ranitidine on indomethacin-induced gastric lesions.

Group	**Dose** (mg/kg)	**Ulcer index** (mm)	**Inhibition** (%)
Control	-	89.17 ± 19.93	-
Ranitidine	100	1.33 ± 0.80 **	99
TI extract	50	85.00 ± 7.96	5
TI extract	100	55.50 ± 4.06 *	38
TI extract	200	25.00 ± 8.76 **	72

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

Significant difference from control (5% Tween80); * $P<0.05$, ** $P<0.001$

3.3.1.3 Restraint water immersion stress-induced gastric lesions

Stress induced lesions in the glandular part of the stomach (Figure 3.12). TI extract at all given doses did not prevent gastric ulcer caused from stress, whereas ranitidine (100 mg/kg) significantly reduced gastric ulcer formation when compared with that of the control group (Figure 3.13, Table 3.11).

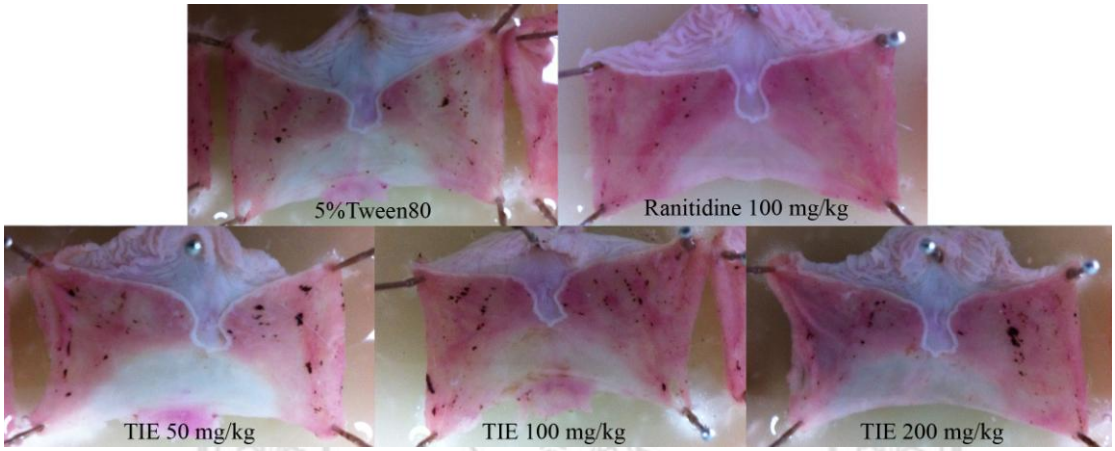

Figure 3.12 Illustrations of gastric ulcer after pretreatment with TI extract and ranitidine compared with the control group on restraint water immersion stress-induced gastric lesions.

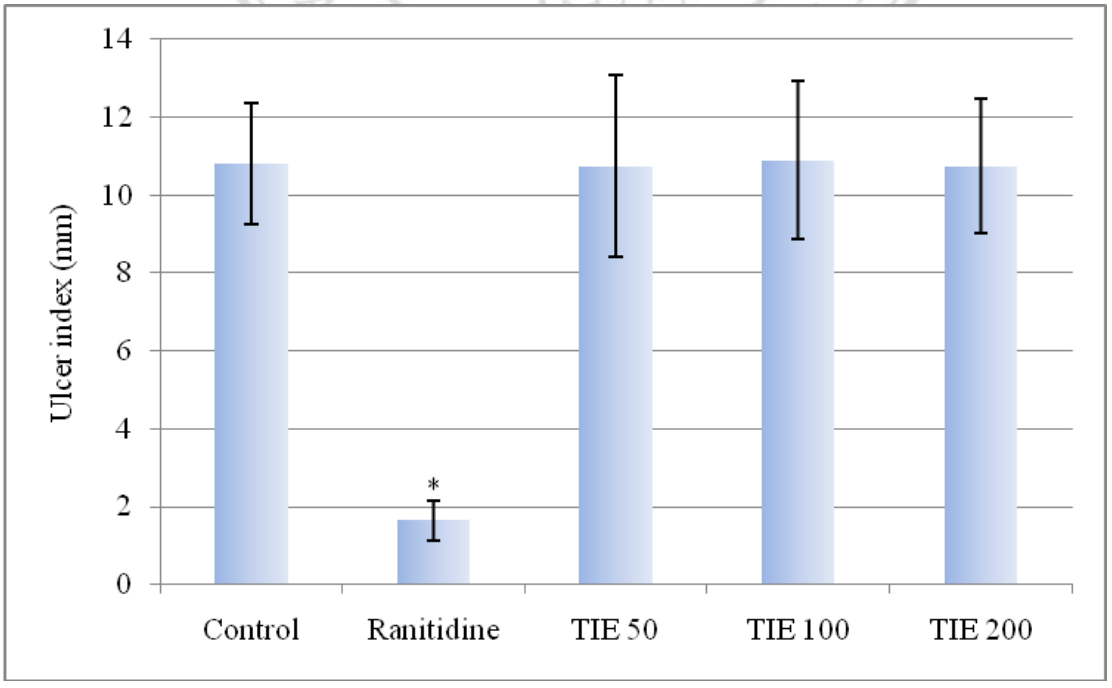

Figure 3.13 Effects of TI extract and ranitidine on restraint water immersion stress-induced gastric lesions. Significant difference from control (5% Tween80); * $P<0.05$.

Table 3.11 Effects of TI extract and ranitidine on restraint water immersion stress-induced gastric lesions.

Group	Dose (mg/kg)	Ulcer index (mm)	Inhibition (%)
Control	-	10.80 ± 1.56	-
Ranitidine	100	1.65 ± 0.51 *	85
TI extract	50	10.75 ± 2.34	0.5
TI extract	100	10.90 ± 2.03	0
TI extract	200	10.75 ± 1.74	0.5

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

Significant difference from control (5% Tween80); * $P<0.05$.

3.3.2 Investigation the mechanisms of anti-gastric ulcer activity

3.3.2.1 Pylorus ligation

The effects of TI extract and ranitidine on gastric volume, gastric pH, and total acidity are shown in Figure 3.14 and Table 3.12. The pylorus ligation caused the decrease of gastric pH resulted from gastric acid secretion that observed in the control group. Ranitidine, an anti-secretory agent, significantly increased gastric pH and reduced gastric volume and total acidity, whereas TI extract at all given doses did not affect gastric volume, gastric pH, and total acidity when compared with those of the control group.

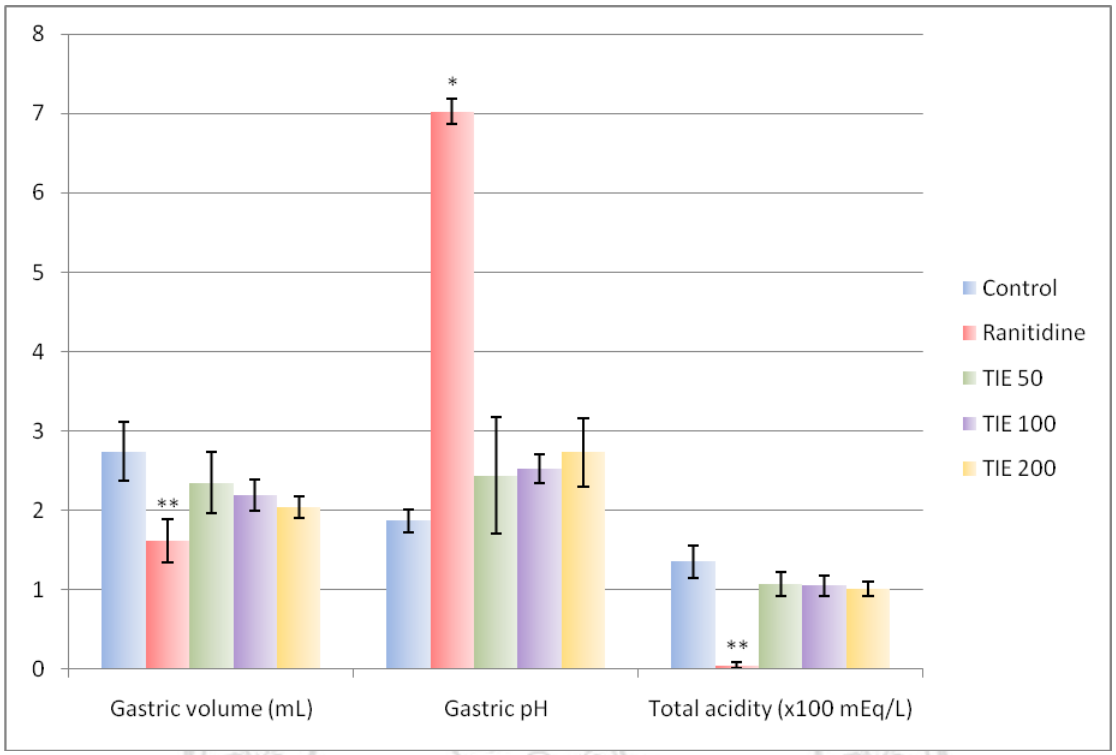

Figure 3.14 Effects of TI extract and ranitidine on pylorus ligation. Significant difference from control (5% Tween80); * $P<0.05$, ** $P<0.001$.

Table 3.12 Effects of TI extract and ranitidine on pylorus ligation.

Group	**Dose** (mg/kg)	**Gastric volume** (ml/100 g body weight)	**Gastric pH**	**Total acidity** (mEq/L)
Control	-	2.74 ± 0.37	1.87 ± 0.14	135.25 ± 19.87
Ranitidine	100	1.62 ± 0.27 *	7.02 ± 0.16 **	5.00 ± 3.42 **
TI extract	50	2.35 ± 0.38	2.44 ± 0.73	107.97 ± 15.13
TI extract	100	2.19 ± 0.20	2.53 ± 0.18	105.36 ± 12.58
TI extract	200	2.04 ± 0.14	2.73 ± 0.43	101.22 ± 9.12

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

Significant difference from control (5% Tween80); * $P<0.05$, ** $P<0.001$.

3.3.2.2 Gastric visible mucus secretion

The effects of TI extract at the dose of 200 mg/kg, ranitidine (100 mg/kg) and misoprostal (100 µg/kg) on gastric wall mucus in EtOH/HCl-induced gastric ulcer model are shown in Figure 1.15 and Table 3.13. Induction of gastric ulcer by EtOH/HCl administration in control group resulted in the significant decrease of the gastric wall mucus amount to 3.40 µg alcian blue/g wet stomach when compared with that of the normal group (5.61 µg alcian blue/g wet stomach). In ranitidine group, the gastric wall mucus amount was not significantly different from that of the control group whereas misoprostol and TI extract prevented the decrease in gastric wall mucus induced by EtOH/HCl. In TI extract-treated group without gastric ulcer induction, the gastric wall mucus of rats tended to increase when compared with that of the normal group but no significant difference was observed (*p* value = 0.095).

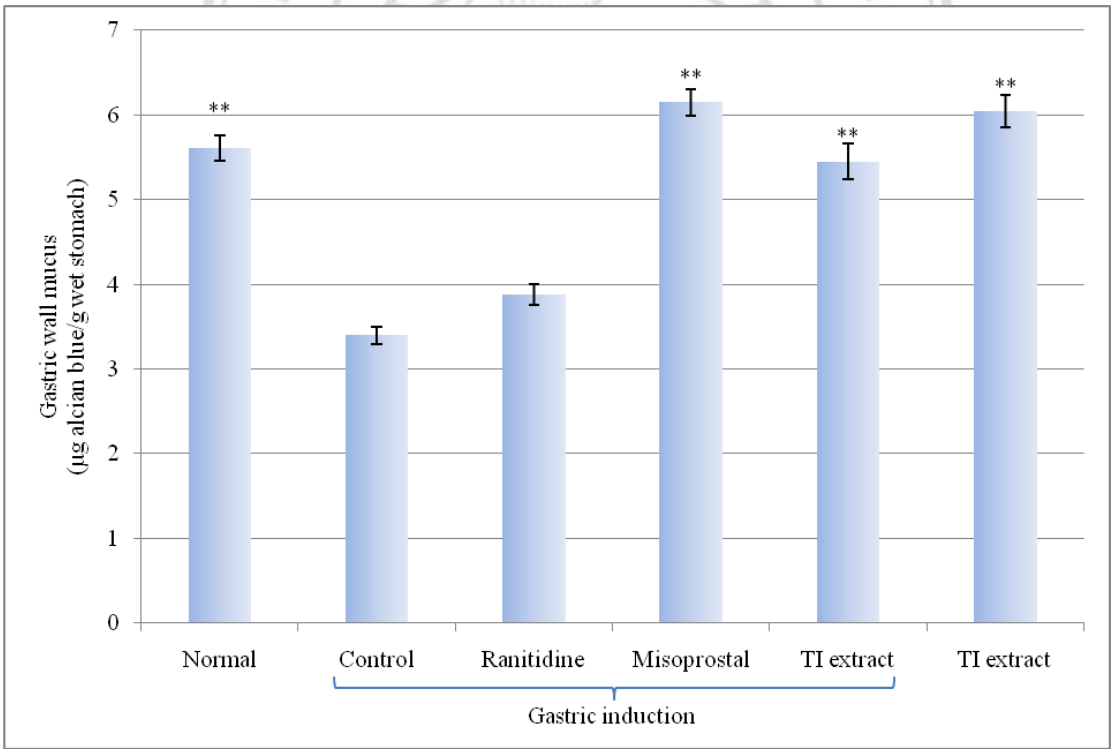

Figure 3.15 Effects of TI extract, ranitidine and misoprostal on gastric wall mucus. Significant difference from control (5% Tween80); ** *P*<0.001.

Table 3.13 Effects of TI extract, ranitidine and misoprostal on gastric wall mucus.

Group	Dose	Gastric wall mucus (µg alcian blue/g wet stomach)
Normal[a]	-	5.61 ± 0.15 **
Control[b]	-	3.40 ± 0.10
Ranitidine[b]	100 mg/kg	3.88 ± 0.12
Misoprostal[b]	100 µg/kg	6.15 ± 0.16 **
TI extract[b]	200 mg/kg	5.45 ± 0.21 **
TI extract[a]	200 mg/kg	6.04 ± 0.19 **

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

[a] No gastric ulcer induction by EtOH/HCl

[b] With gastric ulcer induction by EtOH/HCl

Significant difference from control (5% Tween80); ** $P<0.001$.

3.4 Acute toxicity study

In the acute toxicity study, a single oral administration of TI extract at the dose of 2,000 mg/kg body weight did not produce any mortality, sings of toxicity, changes in general behaviors, or other physiological activities when compared to those of the control group.