CHAPTER 3
RESULTS

3.1 TESTS FOR ANTI-INFLAMMATORY ACTIVITY

3.1.1 EPP-induced ear edema in rats

The inhibitory effect of the standardized water extract of *P. emblica* on EPP-induced rat ear edema is shown in Table 9. In the control group (received DMSO : acetone, 1 : 1), the edema thickness was increased gradually with time and began to decline after 1 h. *P. emblica* water extract at the dose of 1 mg/ear significantly inhibited the edema formation at all determination times. Phenylbutazone (NSAID), at a dose of 1 mg/ear, produced marked anti-edema activity.

3.1.2 AA-induced ear edema in rats

The effects of phenylbutazone (1 mg/ear), phenidone (a dual inhibitor of AA metabolism, 2 mg/ear) and the standardized water extract of *P. emblica* on rat ear edema induced by AA are illustrated in Table 10. AA caused progressive edema formation to reach the peak at the 1st h and then declined rapidly. Only phenidone showed a significant inhibitory effect on the edema formation at assessed time.

Table 9 Effect of the standardized water extract from the fruits of *P. emblica* on EPP-induced ear edema in rats

Group	Dose (mg/ear)	Time after topical application of EPP							
		15 min		30 min		60 min		120 min	
		ED (μm)	EDI (%)	ED (μm)	EDI (%)	ED (μm)	EDI (%)	ED (μm)	EDI (%)
Control	-	91.67 ± 4.77	-	153.33 ± 8.81	-	185.00 ± 13.35	-	128.33 ± 6.54	-
Phenylbutazone	1.0	33.33 ± 11.15*	64	46.67 ± 8.02*	70	68.33 ± 6.54*	63	55.00 ± 5.00*	57
P. emblica	1.0	55.00 ± 7.18*	40	85.00 ± 8.06*	44	86.67 ± 10.22*	53	78.33 ± 11.39*	39

Values are expressed as mean ± S.E.M., n = 6.

* Significantly different from the control group, $p<0.05$.

ED = edema thickness (μm) at time

% EDI = percent edema inhibition of test substance at time

Table 10 Effect of the standardized water extract from the fruits of *P. emblica* on AA-induced ear edema in rats

Group	Dose	60 min after topical application of AA	
	(mg/ear)	ED (μm)	EDI (%)
Control	-	135.00 ± 13.35	-
Phenylbutazone	1.0	118.33 ± 10.14	12
Phenidone	2.0	63.33 ± 5.58*	53
P. emblica	1.0	125.00 ± 12.31	7

Values are expressed as mean ± S.E.M., n = 6.

* Significantly different from the control group, $p<0.05$.

ED = edema thickness (μm) at time

% EDI = percent edema inhibition of test substance at time

1.3 Carrageenan-induced hind paw edema in rats

The inhibitory activities of *P. emblica* water extract and aspirin on carrageenan-induced rat hind paw edema are shown in Table 11. The subplantar injection of carrageenan to the control animals produced a local edema that increased progressively to reach a maximal intensity 3 h after the injection of the phlogistic agent. *P. emblica* water extract, orally given to the rats at the doses of 150, 300 and 600 mg/kg, possessed significant inhibitory effects on carrageenan-induced paw edema at all assessment times. Aspirin, a COX inhibitor at the dose of 300 mg/kg, also exhibited significant edema inhibitory activity.

1.4 Cotton pellet-induced granuloma formation in rats

The inhibitory effects of the standardized water extract of *P. emblica* and reference drugs (aspirin and prednisolone) on the cotton pellet-induced granuloma formation in rats were examined on the eighth day after the daily oral administration of test drugs for 7 days. The inhibitory activities of *P. emblica* water extract and the reference drugs against granuloma formation induced by cotton pellet implantation are shown in Table 12. *P. emblica* water extract at the dose of 600 mg/kg body weight and aspirin (300 mg/kg) did not reduced transudative weight and granuloma formation. The group treated with prednisolone (the steroidal anti-inflammatory drug) at the dose of 5 mg/kg showed a marked inhibition on both parameters.

The body weight gain during the first and the last day of experimental period and the dry weight of thymuses of the rats implanted with cotton pellets are presented in Table 13. Both *P. emblica* water extract and aspirin did not affect the body weight gain and thymus weight of animals. On the contrary, prednisolone significantly reduced those parameters. The effects of test drugs on alkaline phosphatase activity in rats implanted with cotton pellets are shown in Table 14. Serum alkaline phosphatase level of rats in the control group was significantly elevated when compared with that of normal rats (non-implanted rats). The increase in serum alkaline phosphatase caused by cotton pellet implantation into rats was reduced to normal level by the oral administration of prednisolone for 7 days. Whereas, the serum alkaline phosphatase levels of rats receiving *P. emblica* water extract and aspirin were not significantly different from that of the control group.

Table 11 Effect of the standardized water extract from the fruits of *P. emblica* on carrageenan-induced paw edema in rats

Group	Dose (mg/kg)	Time after 1% carrageenan injection					
		1 h		3 h		5 h	
		EV	% EI	EV	% EI	EV	% EI
Control	-	0.26 ± 0.04	-	0.79 ± 0.04	-	0.70 ± 0.03	-
Aspirin	300	0.08 ± 0.01*	69	0.36 ± 0.03*	54	0.30 ± 0.02*	57
P. emblica	150	0.12 ± 0.01*	54	0.52 ± 0.07*	34	0.39 ± 0.06*	44
	300	0.11 ± 0.01*	58	0.46 ± 0.01*	42	0.35 ± 0.04*	50
	600	0.06 ± 0.01*	77	0.36 ± 0.02*	54	0.31 ± 0.03*	56

Values are expressed as mean ± S.E.M., n = 6.

* Significantly different from the control group, $p<0.05$.

EV = edema volume (ml) at time

% EI = percent edema inhibition of test substance at time

Table 12 Effect of the standardized water extract from the fruits of *P. emblica* on granuloma formation and transudation of 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)	GI (%)
Control	-	466.07 ± 9.41	77.42 ± 2.86	388.98 ± 8.34	2.87 ± 0.14	-
Prednisolone	5	350.93 ± 7.15*	63.75 ± 2.17*	287.18 ± 5.75*	2.19 ± 0.11*	23.69
Aspirin	300	449.71 ± 12.17	75.96 ± 2.07	373.75 ± 11.08	2.80 ± 0.10	2.44
P. emblica	600	437.58 ± 17.04	68.92 ± 3.54	368.66 ± 16.22	2.45 ± 0.18	14.63

Values are expressed as mean ± S.E.M., n = 6.

* Significantly different from the control group, $p<0.05$.

GI = percent granuloma inhibition

Table 13 Effect of the standardized water extract from the fruits of *P. emblica* on body weight and thymus weight of cotton pellet-induced granuloma formation in rats

Group	Dose (mg/kg)	Body weight (g)			Dry thymus weight (mg/100g)
		Initial	Final	Gain	
Control	-	240.83 ± 18.23	269.17 ± 15.78	28.33 ± 3.57	47.75 ± 4.50
Prednisolone	5	205.83 ± 17.34	221.67 ± 13.21*	15.83 ± 4.36*	35.14 ± 4.25*
Aspirin	300	234.17 ± 14.28	253.33 ± 14.92	19.17 ± 2.39	50.06 ± 5.95
P. emblica	600	222.50 ± 13.95	253.33 ± 13.14	30.83 ± 4.73	55.63 ± 9.11

Values are expressed as mean ± S.E.M., n = 6.

* Significantly different from the control group, $p<0.05$.

Table 14 Effect of the standardized water extract from the fruits of *P. emblica* on serum alkaline phosphatase activity of cotton pellet-induced granuloma formation in rats

Group	Dose (mg/kg)	Alkaline phosphatase (units/l)	Total protein (g/dl)	Serum alkaline phosphatase activity (U of enz./mg of serum protein x 10^{-4})
Normal	-	109.50 ± 2.79	5.45 ± 0.12	20.13 ± 0.61
Control	-	164.83 ± 10.95	5.27 ± 0.14	31.21 ± 2.03[a]
Prednisolone	5	143.50 ± 7.03	5.63 ± 0.24	25.77 ± 1.85[b]
Aspirin	300	145.33 ± 3.27	5.15 ± 0.13	28.32 ± 1.04
P. emblica	600	153.00 ± 47.90	5.18 ± 0.08	29.49 ± 1.31

Values are expressed as mean ± S.E.M., n = 6.

[a] Significantly different from the normal group, $p<0.05$.

[b] Significantly different from the control group, $p<0.05$.

Normal = non-implanted group

Control = implanted group

3.2 TEST FOR ANALGESIC ACTIVITY

3.2.1 Formalin test in mice

The analgesic test by formalin-induced pain at the right dorsal hind paw of mice was investigated both in the early phase and the late phase using the intensive licking time as a criterion for algesia. Inhibition of licking response of the test drugs in the early phase and late phase of the formalin test is shown in Figure 30.

3.2.1.1 Early phase

The standardized water extract of *P. emblica* showed a significant inhibitory effect on licking response. *P. emblica* water extract at the doses of 150, 300 and 600 mg/kg decreased the licking times with the percent inhibition of 32, 35 and 45, respectively. Aspirin at the dose of 300 mg/kg body weight also slightly but significantly reduced the licking time with percent inhibition of 15. In contrast, morphine, at the dose of 10 mg/kg, completely inhibited the licking response.

2.1.2 Late phase

P. emblica water extract at the doses of 150, 300 and 600 mg/kg markedly decreased the licking time with the percent inhibition of 78, 87 and 96, respectively. Morphine at the dose of 10 mg/kg as well as aspirin at the dose of 300 mg/kg exhibited analgesic effect with complete inhibition of licking response.

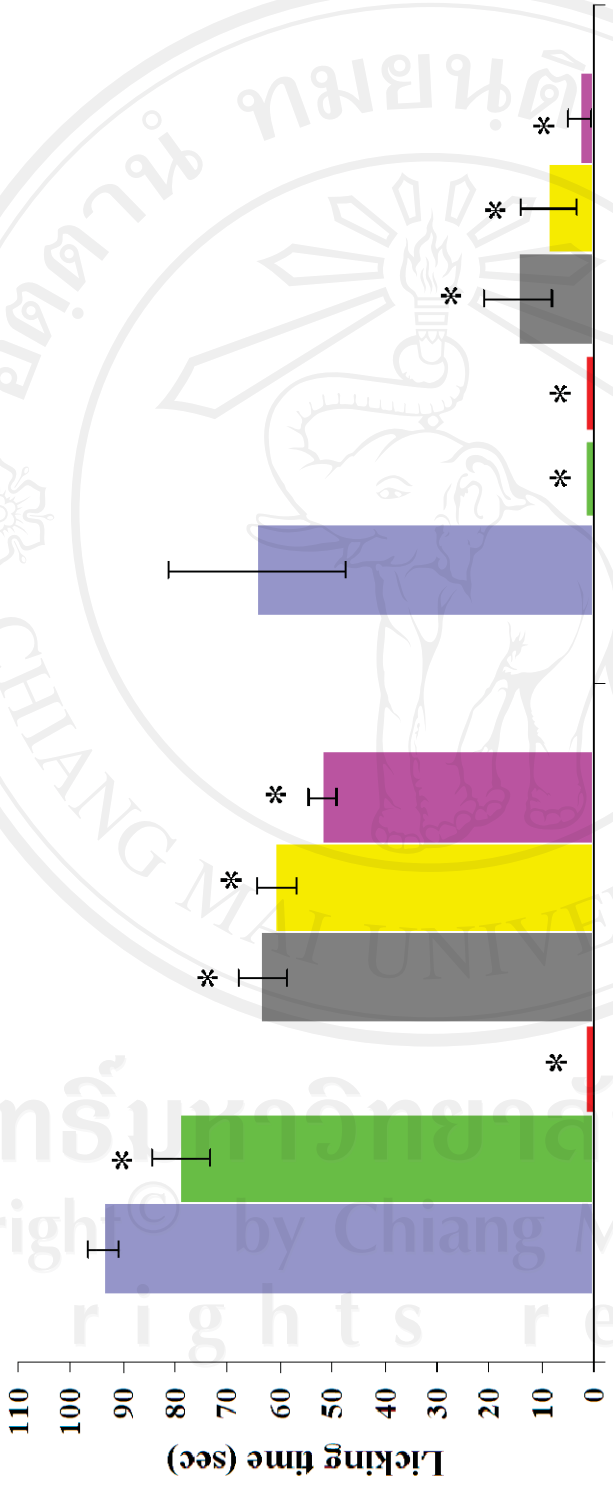


Figure 30 Effect of the standardized water extract from the fruits of *P. emblica* on early phase and late phase of the formalin test in mice (■: control; ■: aspirin 300 mg/kg; ■: morphine 10 mg/kg; ■: *P. emblica* 150 mg/kg; ■: *P. emblica* 300 mg/kg; ■: *P. emblica* 600 mg/kg). * Significantly different from the control group, $p<0.05$.

3.3 TEST FOR ANTIPYRETIC ACTIVITY

3.3.1 Yeast-induced hyperthermia in rats

The antipyretic effect of aspirin and the standardized water extract of *P. emblica* is shown in Figure 31. Eighteen hours after yeast injection, the high rectal temperature of the control group was stable at all assessment times. Aspirin, at the dose of 300 mg/kg, reduced the rectal temperature of the rats when measurement was made at 30, 60, 90 and 120 min after drug administration. Similarly, *P. emblica* water extract at the doses of 300 and 600 mg/kg exhibited significant reduction of the rectal temperature after brewer's yeast injection at all assessment times.

3.4 COX INHIBITORY ACTIVITY

Aspirin, a COX-1 inhibitor, showed 99.98% inhibition of COX-1. In the contrary, celecoxib, a selective COX-2 inhibitor, demonstrated 99.89% inhibition of COX-2. *P. emblica* water extract showed the inhibitory effect on both COX-1 and COX-2. IC_{50} values of *P. emblica* water extract on COX-1 and COX-2 were 54.46 and 34.82 µg/ml, respectively. The IC_{50} ratio of COX-1/COX-2 of *P. emblica* water extract was 1.56.

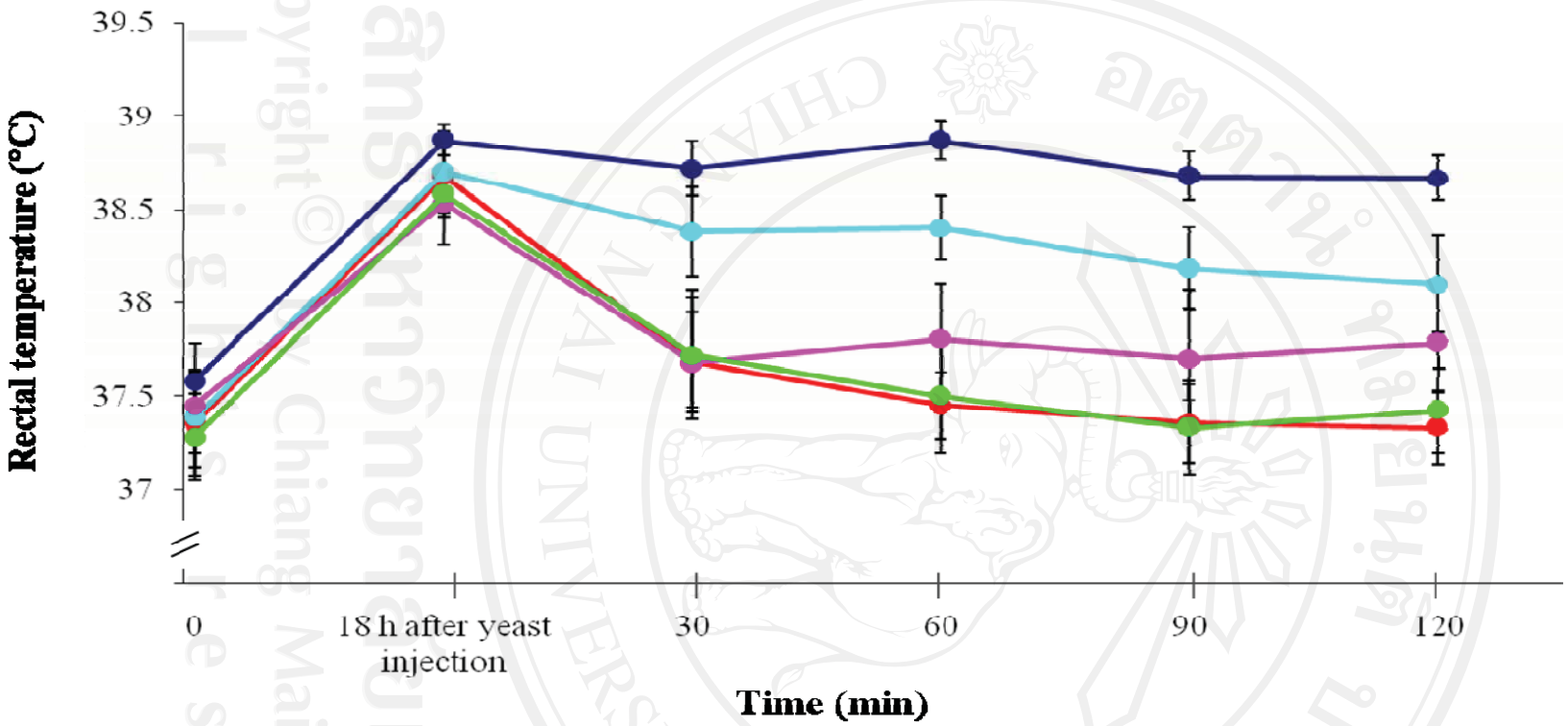


Figure 31 Effect of the standardized water extract from the fruits of *P. emblica* on yeast-induced hyperthermia in rats (●: control; ●: aspirin 300 mg/kg; ●: *P. emblica* 150 mg/kg; ●: *P. emblica* 300 mg/kg; ●: *P. emblica* 600 mg/kg). Rectal temperature of *P. emblica* water extract treated group (300 and 600 mg/kg) and aspirin treated group were significant difference from the control group at all assessment times, $p<0.05$.

3.5 CHONDROPROTECTIVE ACTIVITY

To investigate the effects of *P. emblica* water extract on ECM degradation, porcine cartilage explants were incubated with pro-inflammatory cytokine, IL-1β (5 ng/ml), with a range of doses of *P. emblica* water extract. Diacerein, which is generally used for the treatment of osteoarthritis was also used in this study as the positive control. After 3 days of treatments the culture media were analyzed for S-GAG and HA. Levels of S-GAG and HA released in the control group (without IL-1β) for all samples were set at 100%. The results showed that IL-1β increased the releasing of S-GAG and HA into the culture media for about 179 and 202% of control, respectively (Figures 32 and 33). *P. emblica* water extract at the doses of 25 and 100 μg/ml significantly decreased HA levels in the media, but did not have effect on S-GAG level when compared with those of IL-1β treated group. Diacerein showed similar results to *P. emblica* water extract.

After 21 days of treatments, the cartilage tissue was analyzed for uronic acid and collagen contents. Uronic acid and collagen contents of control group were set at 100%. IL-1β decreased the uronic acid and collagen contents of the cartilage for about 67 and 79% of control, respectively (Figures 34 and 35). *P. emblica* water extract as well as diacerein did not increase the uronic acid and collagen contents in cartilage tissue when compared with those of IL-1β treated group. Diacerein showed similar results to *P. emblica* water extract.

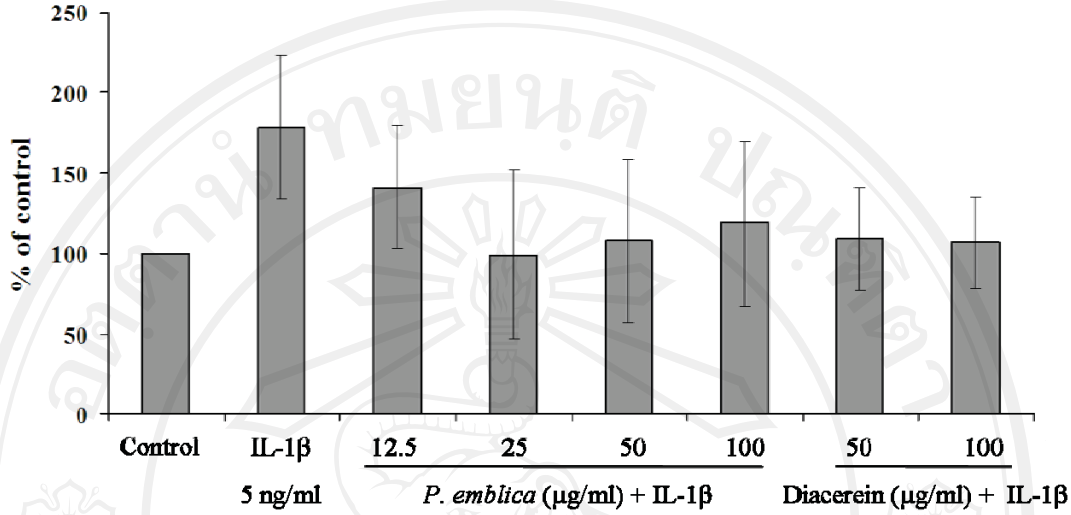


Figure 32 Effect of the standardized water extract from the fruits of *P. emblica* on IL-1β-induced degradation of S-GAG in porcine cartilage explants.

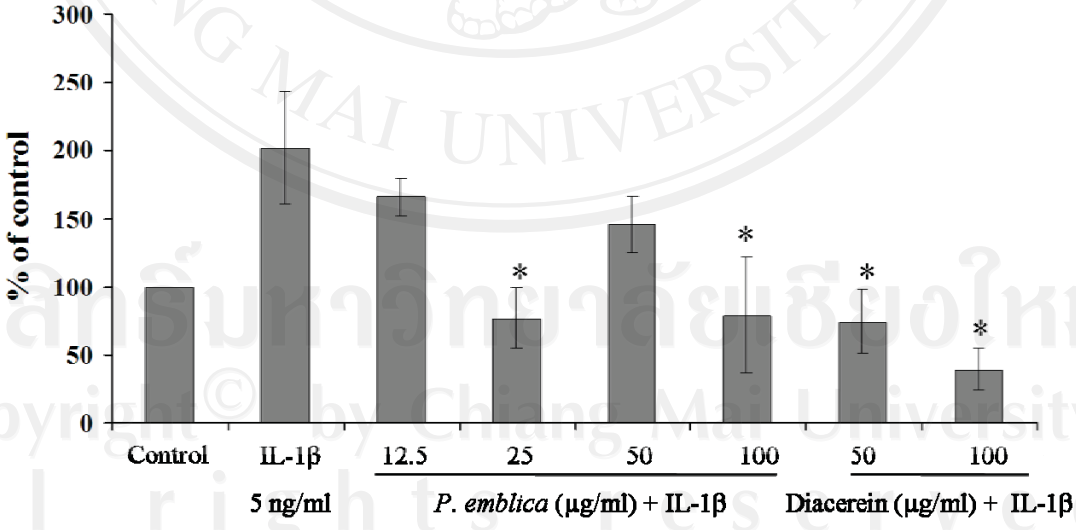


Figure 33 Effect of the standardized water extract from the fruits of *P. emblica* on IL-1β-induced degradation of HA in porcine cartilage explants. *Significantly different from the IL-1β group, $p<0.05$.

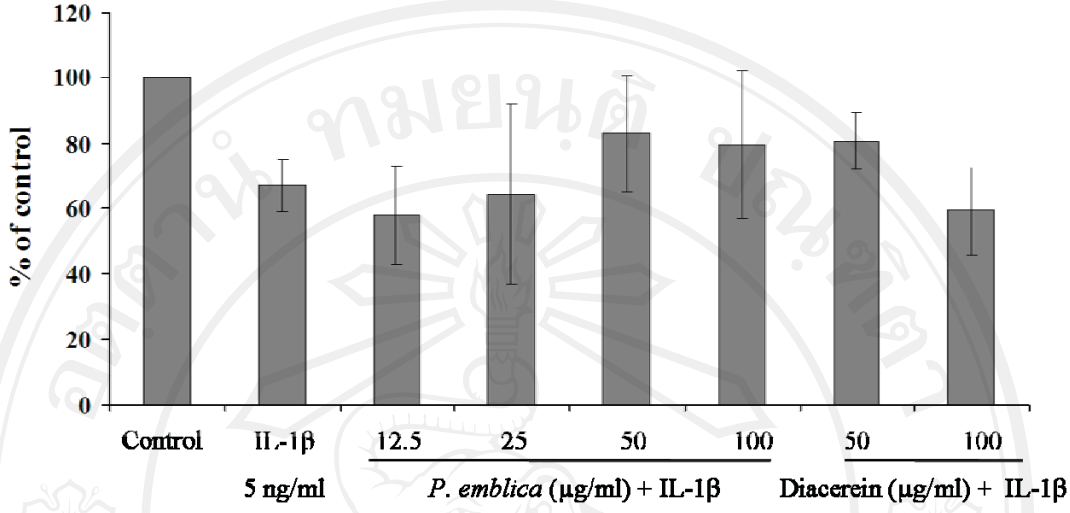


Figure 34 Effect of the standardized water extract from the fruits of *P. emblica* on IL-1β-induced degradation of uronic acid in porcine cartilage explants.

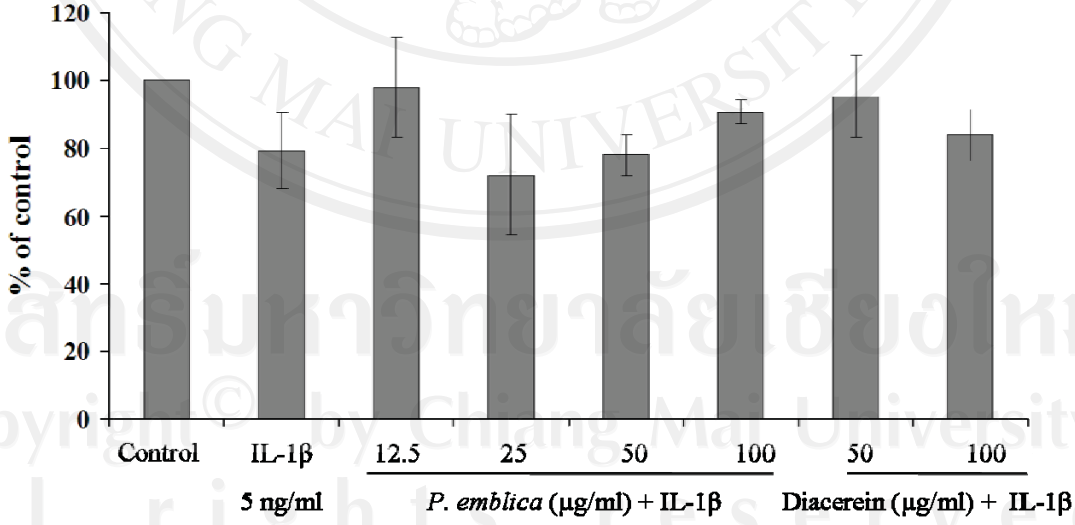


Figure 35 Effect of the standardized water extract from the fruits of *P. emblica* on IL-1β-induced degradation of collagen in porcine cartilage explants.

3.6 TESTS FOR ANTI-ULCEROGENIC ACTIVITY

3.6.1 EtOH/HCl-induced gastric lesions in rats

Oral administration of EtOH/HCl to fasted rats resulted in severe gastric mucosal damage. In the control group, hemorrhagic elongated bands in the glandular segment of stomach were observed (Figure 36). The rats received pretreatment of the water extract of *P. emblica* or cimetidine had less gastric lesions than that of the control group. Table 15 illustrates the protective effect of *P. emblica* water extract against the gastric lesions induced by EtOH/HCl. *P. emblica* water extract, at the doses of 300 and 600 mg/kg, significantly reduced the formation of gastric lesions. Cimetidine, at the dose of 100 mg/kg, also showed a significant anti-ulcerogenic activity.

6.2 Indomethacin-induced gastric lesions in rats

The oral administration of indomethacin caused gastric ulceration in glandular mucosa of the stomach. Most lesions were small and of petechiae lesions as shown in Figure 37. The results of the anti-ulcerogenic effect obtained from the effect of the water extract of *P. emblica* and cimetidine are summarized in Table 16. *P. emblica* water extract (300 and 600 mg/kg) showed statistically significant inhibitory effects on the ulcer lesions. Cimetidine (100 mg/kg) markedly decreased the formation of gastric lesions.

6.3 Restraint water immersion stress-induced gastric lesions in rats

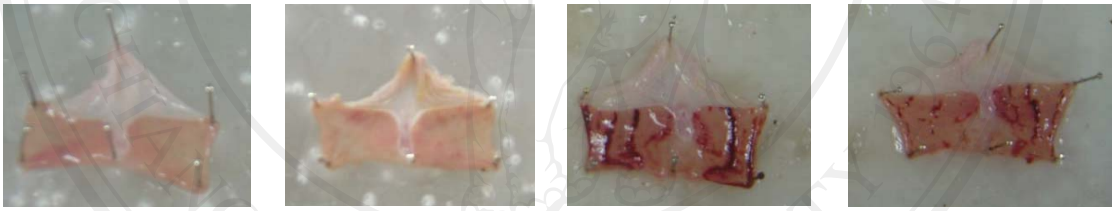
The restraint water immersion stress caused hemorrhagic form of lesion in the glandular part of the stomach as shown in Figure 38. Anti-ulcerogenic activity of the standardized water extract of *P. emblica* and cimetidine is shown in Table 17. *P. emblica* water extract at the doses of 150, 300 and 600 mg/kg showed significant reduction of gastric lesions. Cimetidine, a reference anti-gastric ulcer drug, at the dose of 100 mg/kg exhibited significantly anti-ulcerogenic activity with marked reduction of the gastric lesions.

Table 15 Effect of the standardized water extract from the fruits of *P. emblica* on EtOH/HCl-induced gastric lesions in rats

Group	Gastric lesions (mm)	Inhibition (%)
Normal	0.00 ± 0.00	-
P. emblica 600 mg/kg	0.00 ± 0.00	-
Control + EtOH/HCl	114.33 ± 5.49	-
Cimetidine 100 mg/kg + EtOH/HCl	88.75 ± 12.05*	22
P. emblica 150 mg/kg + EtOH/HCl	101.00 ± 6.08	12
P. emblica 300 mg/kg + EtOH/HCl	90.92 ± 6.59*	20
P. emblica 600 mg/kg + EtOH/HCl	31.00 ± 2.80*	73

Values are expressed as mean ± S.E.M., n = 6.

* Significantly different from the control group, $p<0.05$.



Normal | *P. emblica* 600 mg/kg | Distilled water + EtOH/HCl | Cimetidine 100 mg/kg + EtOH/HCl

P. emblica 150 mg/kg + EtOH/HCl | *P. emblica* 300 mg/kg + EtOH/HCl | *P. emblica* 600 mg/kg + EtOH/HCl

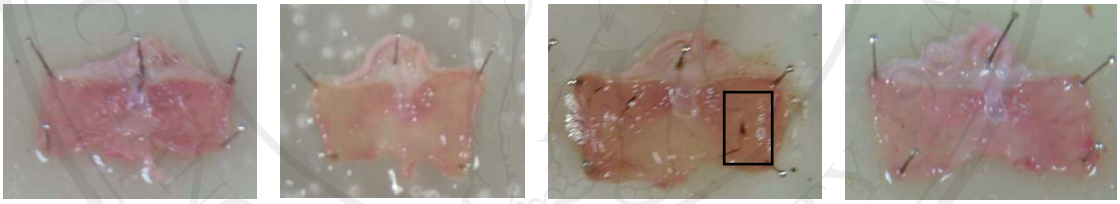
Figure 36 Effects of test substances on gastric lesions in rats induced by EtOH/HCl.

Table 16 Effect of the standardized water extract from the fruits of *P. emblica* on indomethacin-induced gastric lesions in rats

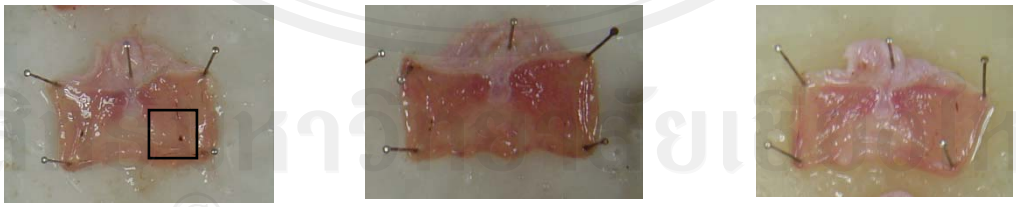
Group	Gastric lesions (mm)	Inhibition (%)
Normal	0.00 ± 0.00	-
P. emblica 600 mg/kg	0.00 ± 0.00	-
Control + Indomethacin	8.05 ± 1.53	-
Cimetidine 100 mg/kg + Indomethacin	0.72 ± 0.25*	91
P. emblica 150 mg/kg + Indomethacin	5.57 ± 0.88	31
P. emblica 300 mg/kg + Indomethacin	4.78 ± 0.63*	41
P. emblica 600 mg/kg + Indomethacin	3.27 ± 0.89*	59

Values are expressed as mean ± S.E.M., n = 6.

*Significantly different from the control group, $p<0.05$.



Normal | *P. emblica* 600 mg/kg | Distilled water + Indomethacin | Cimetidine 100 mg/kg + Indomethacin



P. emblica 150 mg/kg + Indomethacin | *P. emblica* 300 mg/kg + Indomethacin | *P. emblica* 600 mg/kg + Indomethacin

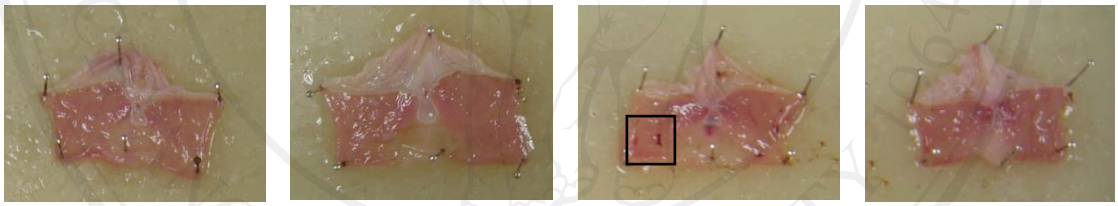
Figure 37 Effects of test substances on gastric lesions in rats induced by indomethacin.

Table 17 Effect of the standardized water extract from the fruits of *P. emblica* on restraint water immersion stress-induced gastric lesions in rats

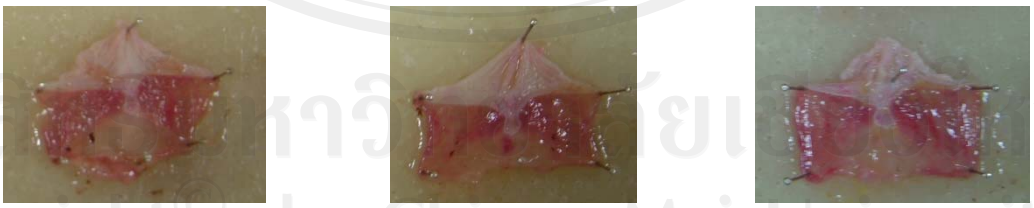
Group	Gastric lesions (mm)	Inhibition (%)
Normal	0.00 ± 0.00	-
P. emblica 600 mg/kg	0.00 ± 0.00	-
Control + Stress	13.68 ± 2.20	-
Cimetidine 100 mg/kg + Stress	2.43 ± 0.74*	82
P. emblica 150 mg/kg + Stress	7.82 ± 2.91*	43
P. emblica 300 mg/kg + Stress	4.90 ± 0.91*	64
P. emblica 600 mg/kg + Stress	2.68 ± 1.11*	80

Values are expressed as mean ± S.E.M., n = 6.

*Significantly different from the control group, $p<0.05$.



Normal | *P. emblica* 600 mg/kg | Distilled water + Stress | Cimetidine 100 mg/kg + Stress



P. emblica 150 mg/kg + Stress | *P. emblica* 300 mg/kg + Stress | *P. emblica* 600 mg/kg + Stress

Figure 38 Effects of test substances on gastric lesions in rats induced by restraint water immersion stress.

3.7 TOXICITY STUDY

3.7.1 Acute oral toxicity

Toxicity evaluation of 5,000 mg/kg of the standardized water extract of *P. emblica* was carried out by observing both body weight gain and internal organ weight as showed in Tables 18 and 19, respectively. In both sexes of rats, body weight gain of treatment rats did not change significantly relative to that of control. For the male treated group, the lung weight was slightly but significantly lower than those of the control group. The internal organs such as brain, lung, heart, liver, spleen, pancreas, adrenal gland, kidney, and sex organs of treated rats showed no pathological abnormality relative to those of the control.

Table 18 Effect of the standardized water extract from the fruits of *P. emblica* on the body weight and weight gain of rats in the acute toxicity test

	Body weight (g)			Weight gain
	Day 0	Day 7	Day 14	on day 14 (g)
Female				
Control	145.80 ± 5.77	181.60 ± 5.03	196.40 ± 4.53	50.60 ± 3.39
P. emblica 5,000 mg/kg	149.20 ± 4.91	180.40 ± 4.69	194.40 ± 4.38	49.20 ± 1.82
Male				
Control	153.20 ± 2.41	208.40 ± 3.08	252.40 ± 4.35	99.20 ± 2.19
P. emblica 5,000 mg/kg	150.40 ± 3.38	204.40 ± 2.96	253.60 ± 4.66	103.20 ± 4.83

Values are expressed as mean ± S.E.M., n = 5.

*Significantly different from the control group, $p<0.05$.

Table 19 Effect of the standardized water extract from the fruits of *P. emblica* on the organ weight (grams/100 grams of rat body weight) of rats in the acute toxicity test

	Control	*P. emblica* 5,000 mg/kg
Female		
Brain	0.87 ± 0.02	0.90 ± 0.02
Lung	0.60 ± 0.02	0.59 ± 0.01
Heart	0.41 ± 0.01	0.41 ± 0.01
Liver	2.71 ± 0.45	3.43 ± 0.27
Spleen	0.29 ± 0.01	0.29 ± 0.01
Adrenal gland	0.02 ± 0.00	0.02 ± 0.00
Kidney	0.41 ± 0.01	0.42 ± 0.01
Ovary	0.03 ± 0.00	0.03 ± 0.00
Uterus	0.26 ± 0.06	0.18 ± 0.01
Male		
Brain	0.73 ± 0.01	0.69 ± 0.01
Lung	0.58 ± 0.02	0.53 ± 0.01*
Heart	0.42 ± 0.01	0.41 ± 0.01
Liver	3.60 ± 0.13	3.40 ± 0.16
Spleen	0.31 ± 0.00	0.32 ± 0.01
Adrenal gland	0.01 ± 0.00	0.01 ± 0.00
Kidney	0.46 ± 0.01	0.44 ± 0.01
Testis	0.56 ± 0.01	0.56 ± 0.01
Epididymis	0.11 ± 0.00	0.11 ± 0.00

Values are expressed as mean ± S.E.M., n = 5.

*Significantly different from the control group, $p<0.05$.

3.7.2 Chronic oral toxicity

3.7.2.1 Body weight

The chronic oral administration of the *P. emblica* water extract (300, 600 and 1,200 mg/kg) resulted in neither change in behaviour nor toxic signs during the experimental period. Body weight and the body weight gain in the treatment groups of female and male rats were significantly lower than those of the control groups (Tables 20 and 21). In rats administered 600 and 1,200 mg/kg doses, body weight of male treatment group significantly decreased on day 180 when compared to that of the control group, whereas the average body weight of rats in the satellite groups was normal until the end of the experiment. In addition, body weight and weight gain of the all male treatment groups significantly decreased on day 270.

7.2.2 Hematological analysis

Regarding hematological examination, significant increase in platelet counts in the female treated rats was observed with the dose of 600 mg/kg/day (Table 22), whereas no change in the male treated rats was found (Table 23). The differential white blood cell counts are listed in Tables 24 and 25. Significant increase in eosinophil was found with the dose of 300 mg/kg/day in female treatment group. Furthermore, significant decrease in neutrophil but increase in lymphocyte were observed in the female satellite group. Significant increase in neutrophil but decrease in lymphocyte were found with the dose of 300 mg/kg/day in male treatment group. However, these values fell within the normal ranges (Appendix A).

7.2.3 Blood chemical analysis

Clinical blood chemistry examination of female and male rats was performed and the results are summarized in Tables 26 and 27, respectively. The results showed significant differences of some biochemical values (glucose, total protein, albumin, SGOT, and ALP) among the experimental groups. Nevertheless, these significant values fell within the normal ranges (Appendix B).

Table 20 Effect of the standardized water extract from the fruits of *P. emblica* on the body weight and weight gain of female rats in the chronic toxicity test

	Dose	Body weight (g)					Weight gain
	(mg/kg)	Day 0	Day 90	Day 180	Day 270	Day 298	on Day 270 (g)
Control	-	166.20 ± 2.37	282.00 ± 5.59	313.50 ± 7.99	352.00 ± 11.62	-	185.80 ± 10.59
P. emblica	300	163.20 ± 3.24	272.50 ± 5.69	299.50 ± 6.68	340.00 ± 8.43	-	176.80 ± 6.88
	600	168.00 ± 3.46	274.50 ± 6.43	297.00 ± 6.96	324.00 ± 9.21	-	156.00 ± 7.23*
	1,200 [a]	165.60 ± 2.00	283.50 ± 3.80	305.00 ± 5.92	335.50 ± 7.43	-	169.90 ± 6.87
	1,200 [b]	167.20 ± 3.84	267.50 ± 6.11	299.50 ± 11.22	324.50 ± 14.84	331.50 ± 13.66	157.30 ± 11.52*

Values are expressed as mean ± S.E.M., n = 10.

* Significantly different from the control group, $p<0.05$.

[a] Receiving the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days.

[b] Satellite group was given the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days followed by no treatment for 28 days.

Table 21 Effect of the standardized water extract from the fruits of *P. emblica* on the body weight and weight gain of male rats in the chronic toxicity test

	Dose	Body weight (g)					Weight gain
	(mg/kg)	Day 0	Day 90	Day 180	Day 270	Day 298	on Day 270 (g)
Control	-	200.80 ± 3.71	458.50 ± 14.12	500.00 ± 10.19	552.50 ± 10.03	-	351.70 ± 9.37
P. emblica	300	202.00 ± 4.53	450.50 ± 8.01	476.50 ± 9.43	506.00 ± 9.51*	-	304.00 ± 8.32*
	600	204.40 ± 4.00	431.00 ± 9.54	459.00 ± 10.02*	489.00 ± 12.60*	-	284.60 ± 14.01*
	1,200 [a]	205.00 ± 3.81	439.00 ± 7.67	473.00 ± 5.12*	503.00 ± 7.08*	-	298.00 ± 6.86*
	1,200 [b]	200.00 ± 3.66	440.50 ± 9.32	476.00 ± 10.77	506.50 ± 11.69*	518.50 ± 9.83	306.50 ± 9.76*

Values are expressed as mean ± S.E.M., n = 10.

* Significantly different from the control group, $p<0.05$.

[a] Receiving the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days.

[b] Satellite group was given the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days followed by no treatment for 28 days.

Table 22 Effect of the standardized water extract from the fruits of *P. emblica* on the hematological values of female rats in the chronic toxicity test

	Control	*P. emblica* (mg/kg)			
		300	600	1,200 [a]	1,200 [b]
Red blood cell ($x10^6/\mu l$)	7.33 ± 0.11	7.36 ± 0.10	7.38 ± 0.12	7.31 ± 0.11	7.39 ± 0.08
Hemoglobin (g/dl)	15.11 ± 0.16	15.01 ± 0.25	15.99 ± 0.86	14.95 ± 0.18	13.28 ± 1.32
Hematocrit (%)	44.50 ± 0.73	44.30 ± 0.60	44.10 ± 0.75	43.80 ± 0.71	44.60 ± 0.58
Mean corpuscular volume (fl)	60.48 ± 0.36	60.01 ± 0.31	59.79 ± 0.30	59.72 ± 0.19	60.21 ± 0.34
Mean corpuscular hemoglobin (pg)	20.63 ± 0.13	20.38 ± 0.14	21.75 ± 1.32	20.47 ± 0.18	19.88 ± 0.16
Mean corpuscular hemoglobin concentration (g/dl)	34.10 ± 0.35	33.96 ± 0.25	36.36 ± 2.19	34.30 ± 0.38	32.99 ± 0.18
Platelet ($x10^5/\mu l$)	6.28 ± 0.41	6.05 ± 0.46	7.38 ± 0.23*	7.08 ± 0.47	6.69 ± 0.17

Values are expressed as mean ± S.E.M., n = 10.

* Significantly different from the control group, $p<0.05$.

[a] Receiving the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days.

[b] Satellite group was given the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days followed by no treatment for 28 days.

Table 23 Effect of the standardized water extract from the fruits of *P. emblica* on the hematological values of male rats in the chronic toxicity test

	Control	*P. emblica* (mg/kg)			
		300	600	1,200 [a]	1,200 [b]
Red blood cell ($x10^6/\mu l$)	8.21 ± 0.11	8.16 ± 0.11	8.31 ± 0.21	8.32 ± 0.07	8.26 ± 0.12
Hemoglobin (g/dl)	15.96 ± 0.19	15.94 ± 0.18	15.92 ± 0.40	16.00 ± 0.11	15.87 ± 0.11
Hematocrit (%)	48.20 ± 0.51	47.80 ± 0.53	48.00 ± 1.19	48.40 ± 0.30	48.60 ± 0.60
Mean corpuscular volume (fl)	58.49 ± 0.32	58.53 ± 0.24	57.94 ± 0.39	58.02 ± 0.26	58.96 ± 0.34
Mean corpuscular hemoglobin (pg)	19.43 ± 0.17	19.56 ± 0.11	19.16 ± 0.12	19.25 0.13	19.24 ± 0.18
Mean corpuscular hemoglobin concentration (g/dl)	33.24 ± 0.18	33.39 ± 0.12	33.07 ± 0.14	33.16 ± 0.15	32.64 ± 0.21*
Platelet ($x10^5/\mu l$)	7.94 ± 0.33	7.44 ± 0.25	8.28 ± 0.26	8.28 ± 0.26	8.00 ± 0.27

Values are expressed as mean ± S.E.M., n = 10.

* Significantly different from the control group, $p<0.05$.

[a] Receiving the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days.

[b] Satellite group was given the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days followed by no treatment for 28 days.

Table 24 Effect of the standardized water extract from the fruits of *P. emblica* on the differential white blood cell count values of female rats in the chronic toxicity test

	Control	*P. emblica* (mg/kg)			
		300	600	1,200 [a]	1,200 [b]
White blood cell ($x10^3/\mu l$)	2.30 ± 0.21	2.02 ± 0.23	3.12 ± 0.25	2.64 ± 0.27	2.70 ± 0.46
Neutrophil (%)	22.90 ± 1.97	19.50 ± 1.97	24.80 ± 2.23	23.60 ± 2.00	15.00 ± 1.16*
Lymphocyte (%)	65.40 ± 2.97	65.70 ± 2.55	64.20 ± 1.77	64.50 ± 2.02	72.90 ± 0.98*
Monocyte (%)	8.30 ± 0.67	9.50 ± 0.82	8.60 ± 0.48	8.60 ± 0.54	9.30 ± 0.39
Eosinophil (%)	3.40 ± 0.75	5.30 ± 0.94*	2.40 ± 0.27	3.30 ± 0.56	2.80 ± 0.44
Basophil (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Values are expressed as mean ± S.E.M., n = 10.

* Significantly different from the control group, $p<0.05$.

[a] Receiving the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days.

[b] Satellite group was given the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days followed by no treatment for 28 days.

Table 25 Effect of the standardized water extract from the fruits of *P. emblica* on the differential white blood cell count values of male rats in the chronic toxicity test

	Control	*P. emblica* (mg/kg)			
		300	600	1,200 [a]	1,200 [b]
White blood cell ($x10^3/\mu l$)	3.51 ± 0.36	3.78 ± 0.36	4.37 ± 0.39	3.95 ± 0.28	4.07 ± 0.38
Neutrophil (%)	24.20 ± 1.74	31.70 ± 2.62*	28.10 ± 3.34	28.60 ± 1.96	22.60 ± 0.87
Lymphocyte (%)	65.70 ± 1.74	57.50 ± 2.17*	61.70 ± 2.97	60.60 ± 2.35	67.30 ± 1.44
Monocyte (%)	7.00 ± 0.61	8.30 ± 1.21	7.90 ± 0.69	8.90 ± 0.82	6.80 ± 0.65
Eosinophil (%)	3.10 ± 0.72	2.50 ± 0.60	2.30 ± 0.47	1.90 ± 0.28	3.30 ± 0.56
Basophil (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Values are expressed as mean ± S.E.M., n = 10.

* Significantly different from the control group, $p<0.05$.

[a] Receiving the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days.

[b] Satellite group was given the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days followed by no treatment for 28 days.

Table 26 Effect of the standardized water extract from the fruits of *P. emblica* on the clinical blood chemistry values of female rats in the chronic toxicity test

	Control	*P. emblica* (mg/kg)			
		300	600	1,200 [a]	1,200 [b]
Glucose (mg/dl)	136.50 ± 4.98	129.40 ± 2.92	134.80 ± 3.66	129.00 ± 4.53	130.10 ± 3.32
BUN (mg/dl)	18.50 ± 0.40	18.30 ± 0.47	19.20 ± 0.71	18.50 ± 0.73	17.50 ± 0.90
Creatinine (mg/dl)	0.39 ± 0.01	0.36 ± 0.02	0.36 ± 0.02	0.37 ± 0.01	0.41 ± 0.01
Total protein (g/dl)	5.80 ± 0.12	5.71 ± 0.08	5.84 ± 0.15	5.93 ± 0.09	5.99 ± 0.08
Albumin (g/dl)	3.72 ± 0.07	3.79 ± 0.07	3.74 ± 0.10	3.77 ± 0.08	4.30 ± 0.07*
Total bilirubin (mg/dl)	0.20 ± 0.01	0.21 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.18 ± 0.01
Direct bilirubin (mg/dl)	0.08 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.10 ± 0.00	0.06 ± 0.01
SGOT (U/l)	91.80 ± 4.72	96.00 ± 6.14	96.30 ± 3.88	92.40 ± 4.90	87.10 ± 4.81
SGPT (U/l)	37.10 ± 3.37	37.20 ± 3.69	40.60 ± 2.39	38.60 ± 3.18	38.30 ± 4.93
ALP (U/l)	24.10 ± 1.16	28.70 ± 2.46	31.90 ± 3.27	34.00 ± 4.78*	32.10 ± 1.78

Values are expressed as mean ± S.E.M., n = 10.

* Significantly different from the control group, $p<0.05$.

[a] Receiving the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days.

[b] Satellite group was given the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days followed by no treatment for 28 days.

Table 27 Effect of the standardized water extract from the fruits of *P. emblica* on the clinical blood chemistry values of male rats in the chronic toxicity test

	Control	*P. emblica* (mg/kg)			
		300	600	1,200 [a]	1,200 [b]
Glucose (mg/dl)	127.40 ± 2.58	126.80 ± 3.23	135.60 ± 2.63	135.00 ± 3.02	148.00 ± 3.23*
BUN (mg/dl)	21.80 ± 0.55	22.40 ± 0.58	21.00 ± 0.60	22.20 ± 0.53	20.90 ± 0.82
Creatinine (mg/dl)	0.41 ± 0.01	0.43 ± 0.01	0.43 ± 0.03	0.41 ± 0.01	0.41 ± 0.03
Total protein (g/dl)	5.53 ± 0.12	5.47 ± 0.11	5.68 ± 0.19	5.80 ± 0.16	6.37 ± 0.07*
Albumin (g/dl)	3.44 ± 0.04	3.51 ± 0.05	3.44 ± 0.07	3.53 ± 0.06	4.14 ± 0.05*
Total bilirubin (mg/dl)	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.14 ± 0.02	0.12 ± 0.01
Direct bilirubin (mg/dl)	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.03 ± 0.01
SGOT (U/l)	103.60 ± 5.48	101.70 ± 6.27	98.00 ± 8.02	110.70 ± 5.37	162.00 ± 4.60*
SGPT (U/l)	53.80 ± 4.22	45.60 ± 3.35	50.20 ± 5.40	49.20 ± 6.87	56.10 ± 4.26
ALP (U/l)	49.30 ± 1.67	46.60 ± 0.94	53.90 ± 4.07	47.60 ± 1.54*	55.20 ± 2.21

Values are expressed as mean ± S.E.M., n = 10.

* Significantly different from the control group, $p<0.05$.

[a] Receiving the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days.

[b] Satellite group was given the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days followed by no treatment for 28 days.

3.7.2.4 Organ weight and histopathology

As shown in Table 28, the satellite female group showed significant increase in the heart, liver and pancreas weight. The organ weight of the male group is listed in Table 29. The brain weight significantly increased in the treatment group with the dose of 600 mg/kg/day. At the dose of 1,200 mg/kg/day, significant weight increase of the pancreas was found. Besides, the satellite male group showed significant increase in the heart, liver and kidney weight. Moreover, all doses of *P. emblica* water extract showed significant increase in testis weight.

The general appearances and the internal organs of rats receiving the extract showed normal structure, size, weight, shape, color, and texture. All intestinal organs of the randomly selection animals were sampled for tissue section. The 4 micron sections of these organs were stained with routine hematoxylin and eosin for histologic examination (Figures 39-42). The brain was unremarkably preserved with intact neuronal layers nuclei. The spinal cord was unremarkable. The heart was composed of intact myocardial fiber without either cardiac hypertrophy or infarcts. Neither fatty change nor fat infiltration was noted. Both lungs were intact, some with mild congestion. The lung parenchyma was unremarkable and lobular architectures were preserved. The liver was normal. The liver cell cords were well aligned with patent sinusoids and intact portal tracts. Neither liver cell destruction nor inflammatory reaction was observed. Minimal congestion of liver and kidneys was found. The kidneys were also normal with unremarkable glomeruli and well aligned renal tubules. Neither glomerular destruction nor tubular necrosis was noted. Adrenal glands were well preserved. They were composed of intact cellular layers with unremarkable capsules. The testes and the ovaries were well preserved with intact spermatogenesis and formation of corpora. The mucosa of stomach, duodenum, and small intestine were unremarkable. The muscular wall and the subserosa were intact. The serosal surface was smooth. Neither hemorrhage nor tissue necrosis was seen. The pancreatic acini and the endocrine islets were intact. Spleen showed no pathologic conditions. The white pulps were remained in normal architectures. Thymus glands were composed of lymphoepithelial lobules. Neither abnormal cellular infiltration nor destruction was noted.

Table 28 Effect of the standardized water extract from the fruits of *P. emblica* on the organ weight (grams/100 grams of rat body weight) of female rats in the chronic toxicity test

	Control	*P. emblica* (mg/kg)			
		300	600	1,200 [a]	1,200 [b]
Brain	0.55 ± 0.02	0.56 ±0.01	0.59 ± 0.02	0.56 ± 0.02	0.57 ± 0.02
Lung	0.48 ± 0.01	0.49 ± 0.01	0.51 ± 0.02	0.47 ± 0.01	0.51 ± 0.02
Heart	0.36 ± 0.01	0.34 ± 0.00	0.35 ± 0.01	0.35 ± 0.01	0.42 ± 0.01*
Liver	2.26 ± 0.07	2.38 ± 0.05	2.30 ± 0.05	2.40 ± 0.06	2.67 ± 0.10*
Spleen	0.23 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.23 ± 0.01
Pancreas	0.39 ± 0.03	0.36 ± 0.02	0.42 ± 0.03	0.42 ± 0.02	0.49 ± 0.06*
Adrenal gland	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00
Kidney	0.34 ± 0.01	0.34 ± 0.01	0.36 ± 0.01	0.35 ± 0.01	0.36 ± 0.01
Ovary	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.00
Uterus	0.21 ± 0.02	0.22 ± 0.03	0.18 ± 0.01	0.24 ± 0.04	0.20 ± 0.02

Values are expressed as mean ± S.E.M., n = 10.

* Significantly different from the control group, $p<0.05$.

[a] Receiving the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days.

[b] Satellite group was given the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days followed by no treatment for 28 days.

Table 29 Effect of the standardized water extract from the fruits of *P. emblica* on the organ weight (grams/100 grams of rat body weight) of male rats in the chronic toxicity test

	Control	*P. emblica* (mg/kg)			
		300	600	1,200 [a]	1,200 [b]
Brain	0.38 ± 0.01	0.39 ± 0.01	0.41 ± 0.01*	0.39 ± 0.01	0.38 ± 0.01
Lung	0.42 ± 0.02	0.48 ± 0.05	0.43 ± 0.03	0.41 ± 0.02	0.42 ± 0.02
Heart	0.29 ± 0.01	0.30 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.33 ± 0.01*
Liver	2.55 ± 0.04	2.59 ± 0.08	2.52 ± 0.09	2.67 ± 0.08	2.84 ± 0.07*
Spleen	0.19 ± 0.00	0.19 ± 0.00	0.18 ± 0.01	0.19 ± 0.01	0.19 ± 0.01
Pancreas	0.29 ± 0.02	0.32 ± 0.03	0.30 ± 0.01	0.35 ± 0.02*	0.32 ± 0.03
Adrenal gland	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Kidney	0.29 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.33 ± 0.01*
Testis	0.36 ± 0.01	0.39 ± 0.01*	0.40 ± 0.01*	0.41 ± 0.00*	0.39 ± 0.01*
Epididymis	0.16 ± 0.00	0.18 ± 0.00	0.17 ± 0.01	0.17 ± 0.00	0.17 ± 0.01

Values are expressed as mean ± S.E.M., n = 10.

* Significantly different from the control group, $p<0.05$.

[a] Receiving the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days.

[b] Satellite group was given the water extract of *P. emblica* at 1,200 mg/kg daily over 270 days followed by no treatment for 28 days.

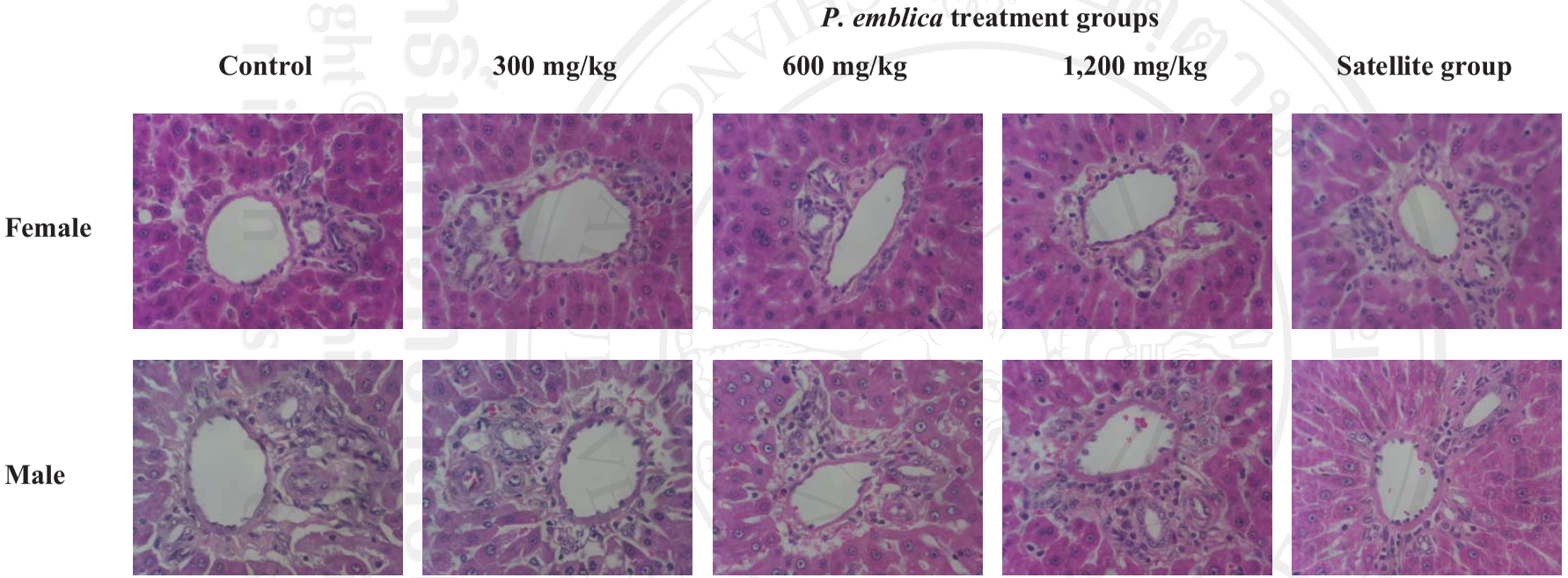


Figure 39 Histopathology of rat portal triad in control and *P. emblica* treatment groups. Section was stained with haematoxylin and eosin. No significant damage was detected in any treatment group (the 40x magnifications).

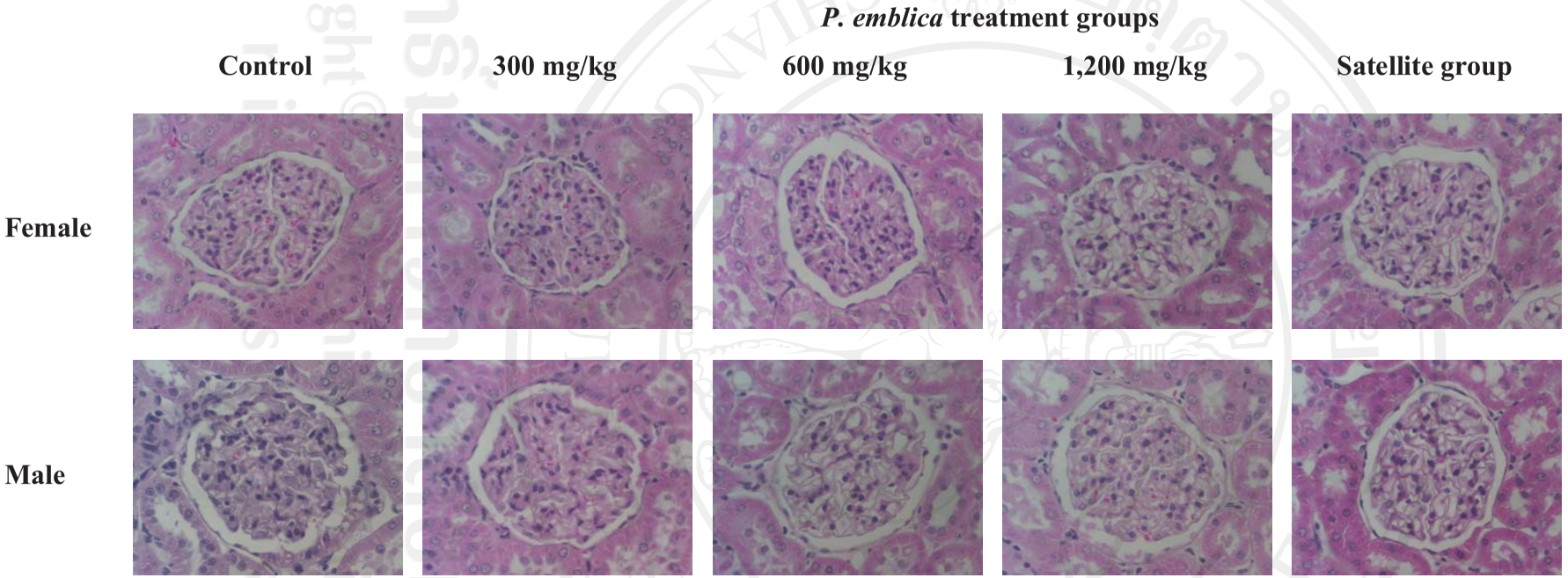


Figure 40 Histopathology of rat renal corpuscles (glomerulus and Bowman's capsule) in control and *P. emblica* treatment groups. Section was stained with haematoxylin and eosin. No significant damage was detected in any treatment group (the 40x magnifications).

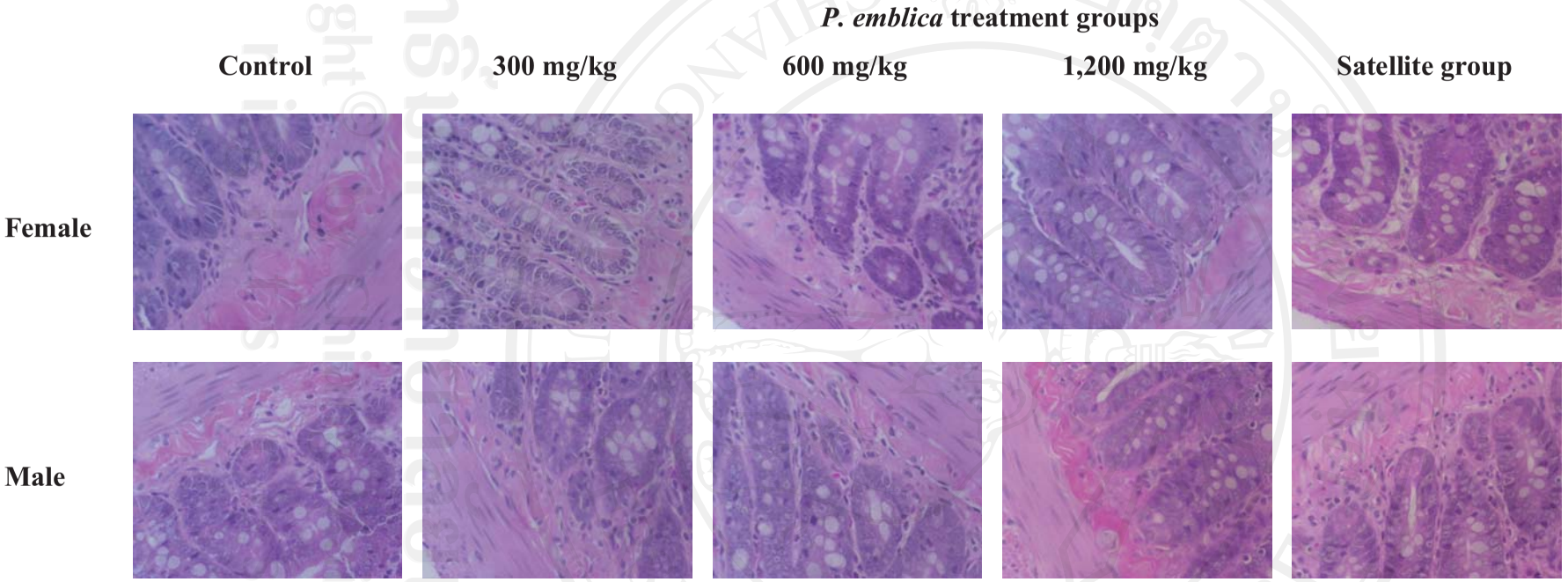


Figure 41 Histopathology of rat small intestine in control and *P. emblica* treatment groups. Section was stained with haematoxylin and eosin. No significant damage was detected in any treatment group (the 40x magnifications).

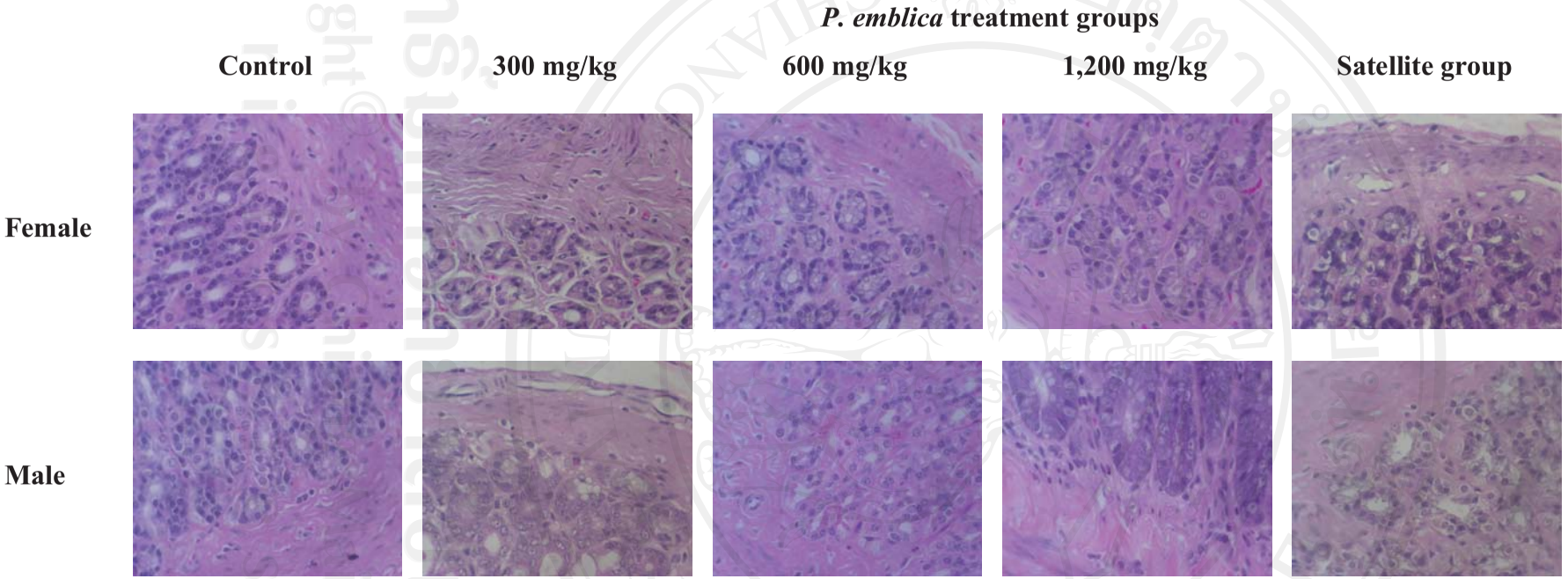


Figure 42 Histopathology of rat stomach in control and *P. emblica* treatment groups. Section was stained with haematoxylin and eosin. No significant damage was detected in any treatment group (the 40x magnifications).