CHAPTER 3

RESULTS

3.1 ANTI-INFLAMMATORY ACTIVITY

3.1.1 Effect of ML extract and indomethacin on carrageenin-induced hind paw edema in rats.

The effect of ML extract against carrageenin-induced hind paw edema is shown in Table 2. In the control group, the edema volume of rat paw was found to increase gradually and reached its peak at 3 h after carrageenin injection. Indomethacin, a COX–inhibitor at the dose of 10 mg/kg markedly reduced the paw edema caused by carrageenin injection with percentages of inhibition of 65, 70, and 63 at the 1st, 3rd, and 5th h, respectively. The ML extract, given orally, significantly reduced the carrageenin-induced edema formation of the rat paw. The antiedematous effect of the ML extract gradually increased as the doses increased. At the 3rd h after carrageenin injection, the percent edema inhibition of ML extract at doses of 100, 200, and 400 mg/kg was found to be 38, 48, and 56, respectively.

Table 2. Effects of ML extract and indomethacin on carrageenin-induced hind paw edema in rats.

Group	Dose (mg/kg)	Time after carrageenin injection					
		1 h		3 h		5 h	
		Edema volume (ml)	Edema inhibition (%)	Edema volume (ml)	Edema inhibition (%)	Edema volume (ml)	Edema inhibition (%)
Control	-	0.17 ± 0.024	-	0.61 ± 0.007	-	0.67 ± 0.025	-
Indomethacin	10	0.06 ± 0.002***	65	0.18 ± 0.001***	70	0.25 ± 0.030***	63
ML extract	100	0.12 ± 0.021*	29	0.38 ± 0.042**	38	0.44 ± 0.044 ***	34
	200	0.09 ± 0.016**	47	0.32 ± 0.035***	48	0.42 ± 0.021***	37
	400	0.06 ± 0.008***	65	0.27 ± 0.045***	56	0.39 ± 0.029***	42

Control received 5% Tween 80 only.

Test drugs were orally administered 1 h before carrageenin injection.

The paw volume was measured prior to and 1, 3, 5 h after carrageenin injection.

Values are expressed as mean ± S.E.M. ($N = 6$). Significantly different from control: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

3.1.2 Effect of ML extract, phenidone and indomethacin on AA-induced hind paw edema in rats.

The result of ML extract on AA-induced hind paw edema in rats is demonstrated in Table 3. In the control group, the average paw edema volume at one hour after AA injection increased 0.60 ± 0.031 ml. Indomethacin did not show any significant inhibitory effect on this model. Phenidone, a dual inhibitor of COX and LOX showed significant anti-edema of the rat paw injected with AA. The ML extract at doses of 100, 200, and 400 mg/kg dose-dependently exhibited significant reduction of the paw edema.

Table 3. Effects of ML extract, phenidone and indomethacin on AA-induced hind paw edema in rats.

Group	Dose (mg/kg)	Edema volume (ml)	Edema inhibition (%)
Control	-	0.60 ± 0.031	-
Indomethacin	10	0.55 ± 0.013	8
Phenidone	40	0.38 ± 0.008***	37
ML extract	100	0.48 ± 0.012***	20
	200	0.43 ± 0.013***	28
	400	0.38 ± 0.022***	37

Control received 5% Tween 80 only.
Test drugs were orally administered 2 h before AA injection.
The paw volume was measured prior to and 1 h after AA injection.
Values are expressed as mean ± S.E.M. (*N* = 6).
Significantly different from control: *** *P* < 0.001.

3.1.3 Effect of ML extract, indomethacin and prednisolone on the cotton pellet-induced granuloma formation in rats.

A. Granuloma formation and transudation

The effect of the ML extract and reference drugs (indomethacin and prednisolone) on the granuloma formation and the transudative weight induced by cotton pellet implantation in rats is shown in Table 4. The results indicated that indomethacin (5 mg/kg) and prednisolone (5 mg/kg) markedly decreased the transudative weight when compared with that of the control group. ML extract at a dose of 400 mg/kg slightly but significantly reduced the transudative weight. The inhibitory effects of the test drugs on granuloma formation were found to be correlated to their effects on the transudative weight. Both indomethacin and prednisolone caused pronound inhibitory effects on granuloma formation whereas ML extract slightly but significantly elicited the inhibitory effect on this parameter.

B. The body weight gain and the thymus weight

The body weight gain during the first and the last day of the experimental period and the thymus weights of rats implanted with cotton pellets are presented in Table 5. The body weight gain of control group increased normally. The gain of the weight in rats treated with indomethacin and ML extract were not significantly different from that of the control group. In contrast, prednisolone significantly reduced the gain of the body weight.

The dry thymus weights of rats in indomethacin- and ML extract-treated groups were not significantly different from that of the control group whereas prednisolone significantly reduced the thymus weight of rats.

C. Alkaline phosphatase activity

The effect of ML extract and reference drugs (indomethacin and prednisolone) on alkaline phosphatase activity are shown on Table 6. Alkaline phosphatase level in the serum during the cotton pellet implantation in the control group (25.27x 10^{-4} U of enz/mg of serum protein) was significantly elevated when compared with that of the normal group or non implanted rats (21.94 x 10^{-4} U of enz/mg of serum protein). Oral administration of reference drugs, indomethacin and prednisolone, for seven days normalized the increased alkaline phosphatase level in the serum to normal levels (20.66 x 10^{-4} and 20.92 x 10^{-4} U of enz/mg of serum protein, respectively). The ML extract also decreased the serum alkaline phosphatase activity to normal level (21.05 x 10^{-4} U of enz/mg of serum protein).

D. Evaluation of ulcerogenic effect

The results are demonstrated in Table 7. It was found that indomethacin at the dose of 5 mg/kg and prednisolone at the dose of 5 mg/kg caused gastric ulceration. The lesions of gastric mucosa of rats in the prednisolone-treated group were small ulcers. In contrast, indomethacin produced large and hemorrhagic gastric lesions in 2 of 6 rats, whereas in the other small ulcers were found. The ML extract did not affect the gastric mucosa of the rats.

Table 4. Effects of ML extract, indomethacin and prednisolone 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)	Granuloma inhibition (%)
Control	-	356.47 ± 6.36	57.42 ± 0.99	299.04 ± 5.62	1.87 ± 0.05	-
Indomethacin	5	245.28 ± 4.42***	37.67 ± 0.55***	207.61 ± 4.19***	0.88 + 0.03***	53
Prednisolone	5	251.92 ± 3.48***	36.66 ± 0.42***	212.25 ± 3.34***	0.98 ± 0.02***	45
ML extract	400	314.17 ± 4.63***	52.67 ± 0.98***	261.50 ± 3.71***	1.63 ± 0.05***	13

Control received 5% Tween 80 only.

Test drugs were orally administered for 7 days. The granuloma tissues were dissected out on the 8th day and weight.

Values are expressed as mean ± S.E.M. (N = 6).

Significantly different from control: *** $P < 0.001$.

Table 5. Effects of ML extract, indomethacin and prednisolone on body weight gain and thymus weight of cotton pellet-induced granuloma formation in rats.

Group	Dose (mg/kg)	Body weight (g)			Dry thymus weight (mg/100 g)
		Initial	Final	Gain	
Control	-	196.67 ± 3.13	237.50 ± 5.51	39.17 ± 1.60	45.79 ± 2.40
Indomethacin	5	195.33 + 3.68	232.33 ± 4.80	37.00 ± 2.77	49.13 ± 1.80
Prednisolone	5	191.33 ± 3.17	221.67 ± 4.11	30.33 ± 2.16 *	32.04 + 1.90 ***
ML extract	400	195.00 ± 2.86	233.33 ± 3.78	38.33 ± 2.60	49.45 ± 3.01

Control received 5% Tween 80 only.

Test drugs were orally administered for 7 days. The final body weight and dry thymus weight were recorded.

Values are expressed as mean ± S.E.M. (N = 6).

Significantly different from control: * $P < 0.05$, *** $P < 0.001$.

Table 6. Effects of ML extract, indomethacin and prednisolone on alkaline phosphatase activity in the serum 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	-	123.87 ± 6.63	5.67 ± 0.29	21.94 ± 0.96
Control	-	140.90 ± 1.54	5.60 ± 0.18	25.27 ± 0.74 [a]*
Indomethacin	5	112.68 ± 3.85	5.45 ± 0.12	20.66 ± 0.41 [b]***
Prednisolone	5	122.70 ± 4.74	5.88 ± 0.14	20.92 ± 0.97 [b]***
ML extract	400	126.18 ± 5.54	6.00 ± 0.23	21.05 ± 0.53 [b]***

Normal = non-implanted group; Control = implanted group, received 5% Tween 80 only.

Test drugs were orally administered for 7 days. Serum alkaline phosphatase and total protein were determined on the 8 [th] day.

Values are expressed as mean ± S.E.M. (N = 6).

[a] significant from normal: * $P < 0.05$

[b] significant from control: *** $P < 0.001$

Table 7. Effects of ML extract, indomethacin, and prednisolone on gastric mucosa.

Group	Dose (mg/kg)	Ulcer index
Control	-	0
Indomethacin	5	3
Prednisolone	5	2
ML extract	400	0

Control received 5% Tween 80 only.
Test drugs were orally administered for 7 days.
Gastric ulcers were determined on the 8 th day.
Values are expressed as median (*N* = 6).
Ulcer index

0 = no pathology
1 = mucosal edema and petechiae
2 = one to five small ulcers (1 to 2 mm)
3 = more of five small ulcers or one medium ulcer (3 to 4 mm)
4 = two or more medium ulcers or large ulcers (>4 mm)
5 = perforated ulcers

3.2 ANALGESIC TEST

3.2.1 Effect of ML extract, indomethacin and codeine on the licking response in the formalin test in mice

3.2.1.1 Early phase

The results in Table 8 show that injection of 1% formalin into the dorsal hind paw of mice produced intensive licking at the injected site with marked licking time. Codeine at the dose of 50 mg/kg caused analgesic activity by inhibition of the licking time. Indomethacin (10 mg/kg) also showed inhibitory effect on the time the mice spent in paw licking. ML extract at doses of 20, 40, and 80 mg/kg dose-dependently reduced the licking time when compared to that of the control group. At a dose of 80 mg/kg, ML extract could produce the same inhibitory effect on the time of licking response as indomethacin at a dose of 10 mg/kg did.

Table 8. Inhibitory effects of ML extract, codeine and indomethacin on the early phase of the formalin test in mice.

Group	Dose (mg/kg)	Licking time (sec)	Inhibition of Licking response (%)
Control		98.17 ± 2.61	-
Indomethacin	10	47.67 ± 4.03***	51
Codeine	50	28.83 ± 1.76***	71
ML extract	20	61.83 ± 5.07***	37
	40	51.83 ± 2.63***	47
	80	42.50 ± 2.08***	57

Control received 5% Tween 80 only.
Test drugs were orally administered 1 h before 1% formalin injection.
The licking time was recorded at 0-5 min after formalin injection.
Values are expressed as mean ± S.E.M. (N = 6).
Significantly different from control: *** $P < 0.001$.

3.2.1.2 Late phase

The analgesic activity of the test drugs on the late phase of formalin test is shown in Table 9. Assessment of algesic effect in the late phase was performed 20 min after injection of 1% formalin to the dorsal side of hind paw of mice. The control group showed marked licking time of 74.83 ± 3.05 sec. Indomethacin at the dose of 10 mg/kg and codeine at the dose of 50 mg/kg produced marked analgesic effect by reducing the licking time caused by formalin injection. The ML extract at doses of 20, 40, and 80 mg/kg also gradually reduced the licking time in dose-related manner.

Table 9. Inhibitory effects of ML extract, codeine and indomethacin on the late phase of the formalin test in mice.

Group	Dose (mg/kg)	Licking time (sec)	Inhibition of Licking response (%)
Control		74.83 ± 3.05	-
Indomethacin	10	19.83 ± 2.62***	74
Codeine	50	15.50 ± 4.60***	79
ML extract	20	43.50 ± 3.83***	42
	40	32.67 ± 5.26***	56
	80	25.33 ± 4.06***	66

Control received 5% Tween 80 only.
Test drugs were orally administered 40 min before 1% formalin injection.
The licking time was recorded at 20-30 min after formalin injection.
Values are expressed as mean ± S.E.M. (N = 6).
Significantly different from control: *** $P < 0.001$.

3.3 ANTIPYRETIC TEST

3.3.1 Effect of ML extract and indomethacin on yeast-induced hyperthermia in rats

The antipyretic effect of ML extract and indomethacin is shown in Table 10 and Figure 9. Eighteen hours after yeast injection rectal temperature of all rats raised more than 1 ^{0}C. In control group the rectal temperature was stable, although slightly but non-significantly declined after 120 min. Indomethacin at the oral dose of 10 mg/kg significantly reduced the rectal temperature back to normal within 30 min and lasted for 180 min. The oral administration of ML extract at the dose of 400 mg/kg also decreased the rectal temperature to normal within 30 min after administration and lasted to the last assessment time (180 min).

3.4 ACUTE TOXICITY

A single administration of the ML extract by oral route at the high dose of 5000 mg/kg did not produce mortality or show any signs of toxicity or changes in general behavior or other physiological activities when compared with those of the control group.

On the eight day, all rats were sacrificed to examine gross pathological changes of internal organs, the result showed that there were no detectable abnormalities and no differences between the control and the ML extract-treated group.

Table 10. Effect of ML extract and indomethacin on yeast-induced hyperthermia in rats.

Group	Dose (mg/kg)	Rectal temperature (°C)					
		Before Yeast injection	18 h after yeast injection	Time after medication (min)			
				30	60	120	180
Control	-	37.58 + 0.17	38.70 + 0.15	38.45 + 0.07	38.43 + 0.16	38.07 + 0.13	38.08+ 0.14
Indomethacin	10	37.58 + 0.12	38.76 + 0.13	37.87 + 0.14*	37.28 + 0.13**	36.83 + 0.13***	36.78 + 0.07***
ML extract	400	37.45 + 0.09	38.53 + 0.10	37.81 + 0.23*	37.60 + 0.26**	37.32 + 0.21**	37.23 + 0.20**

Control received 5% Tween 80 only.

Test drugs were orally administered 18 h after yeast injection.

Rectal temperature was measured at 30 min, 1, 2, and 3 h after medication.

Values are expressed as mean ± S.E.M. (N = 6).

Significantly different from the rectal temperature after yeast injection 18 h: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

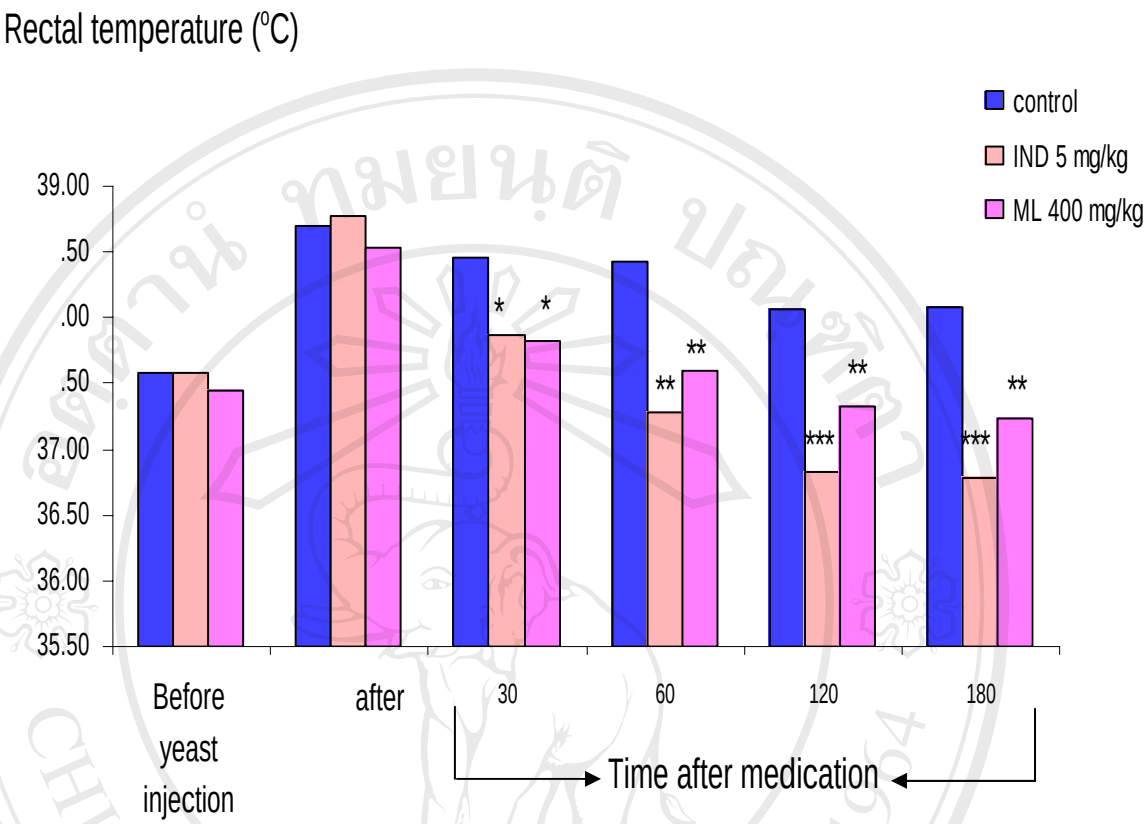

Figure 10. Effects of ML extract and indomethacin on yeast- induced hyperthermia in rats.

Control received 5% Tween 80 only.
Test drugs were orally administered 18 h after yeast injection.
Rectal temperature was measured at 30 min, 1, 2, and 3 h after medication.
Values are expressed as mean ± S.E.M. (N = 6).
Significantly different from the rectal temperature after yeast injection 18 h:
* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.