CHAPTER 3

RESULTS

3.1 Anti-inflammatory study

3.1.1 Effects of DL extract and diclofenac on carrageenin-induced hind paw edema in rats.

Effect of DL extract and diclofenac against carrageenin-induced hind paw edema are shown in Table 3. 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. Diclofenac, a COX–inhibitor at the dose of 10 mg/kg markedly reduced the paw edema caused by carrageenin injection with percentages of inhibition of 83, 80 and 67 at the 1^{st}, 3^{rd}, and 5^{th} h, respectively. The DL extract significantly reduced the carrageenin-induced edema formation of the rat paw. The antiedematous effects of the DL extract were dose dependent. At the 3^{rd} h after carrageenin injection, the percentage of edema inhibition of DL extract at doses of 100, 200, and 400 mg/kg were found to be 40, 58, and 63, respectively.

Table 3. Effects of DL extract and diclofenac on carrageenin-induced hind paw edema in rats.

Group	Dose (mg/kg)	Time after carrageenin injection					
		1 h		3 h		5 h	
		EV (mL)	%EI	EV (mL)	%EI	EV (mL)	%EI
Control	-	0.33±0.03	-	0.69±0.04	-	0.61±0.04	-
Diclofenac	10	0.06±0.02**	83	0.14±0.02**	80	0.20±0.02**	67
DL extract	100	0.21±0.04*	37	0.42±0.05**	40	0.42±0.03*	31
	200	0.17±0.03*	49	0.29±0.02**	58	0.28±0.04**	55
	400	0.09±0.02**	74	0.25±0.04**	63	0.23±0.04**	62

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 *** $P < 0.00$

EV = edema volume (mL), % EI = percent edema inhibition

3.1.2 Effect of DL extract, diclofenac and prednisolone on AA-induced hind paw edema in rats.

Results of DL extract, diclofenac and prednisolone on AA-induced hind paw edema in rats are demonstrated in Table 4. In the control group, the average paw edema volume at one hour after AA injection increased 0.43±0.03 mL. Diclofenac did not show any significant inhibitory effect on this model. Prednisolone, a phospholipase A_2 inhibitors showed significant anti-edema of the rat paw injected with AA. The DL extract at doses of 100, 200, and 400 mg/kg did not possess significant inhibitory effect on AA-induced hind paw edema.

Table 4. Effects of DL extract, diclofenac and prednisolone on AA-induced hind paw edema in rats.

Groups	Dose (mg/kg)	EV (mL)	% EI
Control	-	0.43±0.03	-
Diclofenac	10	0.39±0.02	10
Prednisolone	40	0.27±0.01**	37
DL extract	100	0.43±0.05	0
	200	0.37±0.06	13
	400	0.33±0.04	24

Control received 5% Tween 80 only.
Test drugs were orally administered 2 h before AA injection.
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: ** $P < 0.01$.
EV = edema volume (mL), % EI = percent edema inhibition

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

A. Granuloma formation and transudation

Effect of the DL extract and reference drugs (diclofenac and prednisolone) on the granuloma formation and the transudative weight induced by cotton pellet implantation in rats are shown in Table 5. The results indicated that diclofenac (5 mg/kg) and prednisolone (5 mg/kg) markedly decreased the transudative weight and granuloma formation when compared with that of the control group but DL extract at a dose of 400 mg/kg did not significantly reduced both transudative weight and granuloma formation.

B. Alkaline phosphatase activity

Effects of DL extract and reference drugs (diclofenac and prednisolone) on alkaline phosphatase activity are shown on Table 7. Alkaline phosphatase level in the serum during the cotton pellet implantation in the control group (48.93×10^{-4} U of enz/mg of serum protein) was significantly elevated when compared with that of the normal group or non implanted rats (33.58×10^{-4} U of enz/mg of serum protein). Oral administration of reference drugs, diclofenac and prednisolone, for seven days normalized the increased alkaline phosphatase level in the serum to normal levels (33.54×10^{-4} and 31.16×10^{-4} U of enz/mg of serum protein, respectively). The DL extract did not reduce alkaline phosphatase activity to normal level (48.49×10^{-4} U of enz/mg of serum protein).

C. Body weight gain and the thymus weight

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 6. The body weight of control group increased normally. The gains of the weight in rats treated with diclofenac and DL extract were not significantly different from that of the control group. In contrast, prednisolone significantly reduced the rats body weight gain.

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

D. Evaluation of ulcerogenic effect

Diclofenac 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, diclofenac caused large and hemorrhagic gastric lesions. The DL extract did not affect the gastric mucosa of the treated rats (Table 8).

Table 5. Effects of DL extract, diclofenac 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	-	445.24±30.77	83.61±5.47	372.42±19.91	3.17±0.27	-
Diclofenac	5	295.64±35.33***	60.89±5.52*****	234.75±30.11***	2.02±0.28**	36
Prednisolone	5	282.35±22.50***	54.90±4.47*******	227.20±18.21***	1.74±0.22***	45
DL extract	400	364.42±15.47	79.92±3.86	354.54±12.79	3.16±0.25	0.32

Control received 5% Tween 80 only.

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

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

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

Table 6. Effects of DL extract, diclofenac and prednisolone on alkaline phosphatase activity in the serum of cotton pellet-induced granuloma formation in rats

Group	Dose (mg/kg)	Alkaline phosphatase (U/L)	Total protein (g/dL)	Serum alkaline phosphatase activity (U of enz/mg of serum protein x 10^{-4})
Normal	-	173.50±20.42	5.10±0.22	33.58±2.36*[b]
Control	-	249.17±19.36	5.18±0.18	48.93±5.17*[a]
Diclofenac	5	184.00±9.00	5.50±0.25	33.54±1.80*[b]
Prednisolone	5	172.33±8.32	5.51±0.06	31.16±1.72*[b]
DL extract	400	243.83±10.50	5.17±0.05	45.90±2.21*[a]

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 difference from normal: * $P < 0.05$

[b] significant difference from control: * $P < 0.05$

Table 7. Effects of DL extract, diclofenac and prednisolone 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/100 g)
		Initial	Final	Gain	
Control	-	213.00±4.12	258.00±7.65	45.00±6.21	39.91±3. 51
Dicofenac	5	213.66±4.01	249.33±6.68	35.66±5.982	32.72±1.72
Prednisolone	5	205.00±3.41	214.66±4.94***	9.66±3.48***	19.69±3.20***
DL extract	400	217.00±4.91	267.66±5.29	50.66±38.23	33.39±2.01

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: *** $P < 0.001$.

Table 8. Effects of DL extract, diclofenac, and prednisolone on gastric mucosa.

Group	Dose (mg/kg)	Ulcer index
Control	-	0
Diclofenac	5	3
Prednisolone	5	1
DL 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.

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 study

3.2.1 Effects of DL extract, diclofenac and morphine on the licking response in the formalin test in mice

3.2.1.1 Early phase

The results in Table 9 show that injection of 1% formalin into the dorsal hind paw of mice produced intensive licking at the injected site. Morphine at the dose of 10 mg/kg caused analgesic activity by abolishing the licking time. Diclofenac (5 mg/kg) showed modest and non-significant inhibitory effect on the time the mice spent in paw licking. DL extract at doses of 5, 25, 50, 100 and 200 mg/kg dose-dependently and significantly reduced the licking time when compared to that of the control group.

Table 9. Inhibitory effects of DL extract, diclofenac and morphine on the early phase of the formalin test in mice.

Group	Dose (mg/kg)	Licking time (sec)	Inhibition of licking response (%)
Control	-	100.28±6.13	-
Diclofenac	5	89.40±2.71	11
Morphine	10	0***	100
DL extract	5	82.09±3.65*	18
	25	78.43±6.07*	22
	50	69.16±6.87***	31
	100	51.10±6.31***	49
	200	41.26±8.09***	59

Control received 5% Tween 80 only.

Test drugs were intraperitoneal administered 1 h before 1% formalin injection.

The licking time was recorded at 5-10 min after formalin injection.

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

Significantly different from control: * $P < 0.05$; ** $P < 0.01$,*** $P < 0.001$.

3.2.1.2 Late phase

The analgesic activities of the test drugs on the late phase of formalin test are shown in Table 10. Assessment of analgesic 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 160.79 ± 9.04 sec. Diclofenac at the dose of 5 mg/kg and morphine at the dose of 10 mg/kg produced marked and significant analgesic effects by reducing the licking time caused by formalin injection. DL extract at doses of 5, 25,50,100 and 200 mg/kg also reduced the licking time in dose-related manner.

Table 10. Inhibitory effects of DL extract, diclofenac and morphine on the late phase of the formalin test in mice.

Group	Dose (mg/kg)	Licking time (sec)	Inhibition of licking response (%)
Control	-	160.79±9.04	-
Diclofenac	5	26.06±6.39***	84
Morphine	10	0***	100
DL extract	5	70.84±5.09***	56
	25	44.27±7.37***	72
	50	36.11±6.31***	78
	100	26.11±5.11***	84
	200	19.85±6.43***	88

Control received 5% Tween 80 only.

Test drugs were intraperitoneal 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 study

3.3.1 Effects of DL extract and diclofenac on yeast-induced hyperthermia in rats

The antipyretic effect of DL extract and diclofenac are shown in Table 11 and Figure 11. Eighteen hours after yeast injection rectal temperature of all rats raised more than 1^{o}C. The rectal temperature was stable in control group, although it was non-significantly declined after 120 min. Diclofenac at the oral dose of 10 mg/kg significantly decreased the rectal temperature to normal within 30 min and its antipyretic effect was sustained for at least 180 min. The oral administration of DL extract at the dose of 400 mg/kg also decreased the rectal temperature to normal within 30 min after administration and it antipyretic effect was also equally rapid and long lasting of at least 180 min as was diclofenac.

Table 11. Effects of DL extract and diclofenac 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	-	37.30±0.19	38.95±0.17	38.80±0.18	38.67±0.17	38.60±0.13	38.51±0.14
Diclofenac	10	37.36±0.09	38.68±0.09	37.68±0.18***a***b	37.30±0.15***a***b	37.20±0.11***a***b	37.15±0.12***a***b
DL extract	400	37.13±0.23	38.60±0.11	37.83±0.13**a***b	37.68±0.17**a***b	36.98±0.22***a***b	37.21±0.25***a***b

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).

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

[b] Significantly different from control: *** $P < 0.001$.

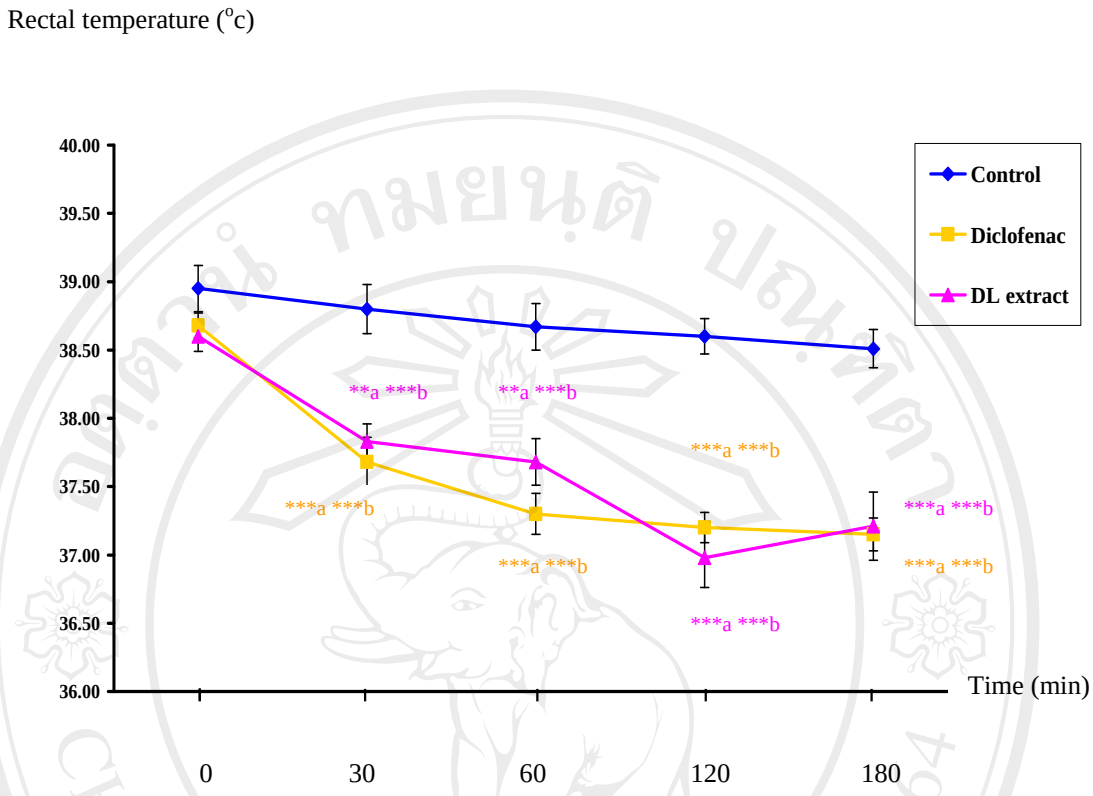

Figure 11. Effects of DL extract and diclofenac on yeast-induced hyperthermia in rats.

Control received 5% Tween 80 only.

Test drugs were orally administered 18 h after yeast injection.

Rectal temperature was measured at 30, 60, 120, and 180min after medication.

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

[a] Significantly different from the rectal temperature after yeast injection 18 h (0 min): ** $P < 0.01$ and *** $P < 0.001$.

[b] Significantly different from control: *** $P < 0.001$.