CHAPTER 3
RESULTS

3.1 Analgesic activity of AP extract

3.1.1 Effects of AP extract and diclofenac on acetic acid-induced writhing response in mice

The inhibitory effects of AP extract and diclofenac on acetic acid-induced writhing response in mice are demonstrated in Table 4. In the test group, all doses of AP extract possessed significant inhibitory effect on acetic acid-induced writhing response in a dose-dependent manner ($R^2 = 0.9643$). Similarly, diclofenac at the dose of 10 mg/kg showed a marked inhibition on writhing response.

3.1.2 Effect of AP extract, diclofenac and codeine on the tail-flick test in rats

The inhibitory effects of AP extract, diclofenac and codeine on tail-flick test in rats are demonstrated in Table 5. The maximal dose of AP extract (600 mg/kg) and diclofenac did not show significant inhibitory effect on the reaction time. In contrast, codeine exhibited marked inhibitory effect on the tail-flick response in rats.

3.2 Anti-inflammatory activity of AP extract

3.2.1 Effects of AP extract and diclofenac on EPP-induced ear edema in rats

The inhibitory effects produced by topical administration of AP extract and diclofenac on EPP-induced ear edema are shown in Table 6. The ear thickness of rats in the control group (received ethanol only) increased gradually and the maximum edema was observed at 30 min after EPP application. Diclofenac at the dose of 3 mg/ear significantly reduced the edema formation at all assessment times. Similarly, AP extract at the dose of 3 mg/ear produced significant but weaker inhibitory activity on edema formation at all determination times than diclofenac.

Table 4 Effects of AP extract and diclofenac on acetic acid-induced writhing response in mice

Group	Dose (mg/kg)	No. of writhes	Inhibition of writhing response (%)
Control	-	24.00 ± 2.11	-
Diclofenac	10	5.67 ± 1.90*	76
AP extract	150	14.50 ± 2.53*	40
AP extract	300	11.50 ± 1.43*	52
AP extract	600	5.83 ± 1.14*	76

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

Significantly different from the control group: * $p < 0.05$

Table 5 Effects of AP extract, diclofenac and codeine on the tail-flick test in rats

Group	Dose (mg/kg)	T_c (sec)	T_t (sec)	% inhibition
Control	-	2.40 ± 0.11	2.85 ± 0.22	-
Diclofenac	10	2.70 ± 0.22	3.18 ± 0.28	7
Codeine	200	2.87 ± 0.18	9.00 ± 0.55*	86
AP extract	600	2.52 ± 0.20	3.67 ± 0.71	15

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

Significantly different from the control group: * $p < 0.05$

T_c = control reaction time; T_t = reaction time after receiving of test drug

Table 6 Effects of AP extract and diclofenac on EPP-induced ear edema in rats

Group	Dose (mg/ear)	Time after topical application of EPP							
		15 min		30 min		1 h		2 h	
		ED (μm)	% EI	ED (μm)	% EI	ED (μm)	% EI	ED (μm)	% EI
Control	-	188.33 ± 10.78	-	208.33 ± 7.03	-	136.67 ± 6.67	-	96.67 ± 7.15	-
Diclofenac	3	36.67 ± 4.94*	81	50.00 ± 2.58*	76	30.06 ± 5.16*	78	23.33 ± 4.97*	76
AP extract	3	63.33 ± 4.22*	66	60.00 ± 5.77*	71	43.33 ± 7.60*	68	35.00 ± 6.19*	64

Results are expressed as mean ± S.E.M. (N of ears = 6)

Significantly different from the control group: * $p < 0.05$

ED = ear edema; % EI = percent edema inhibition

3.2.2 Effects of AP extract and diclofenac on carrageenin-induced hind paw edema in rats

The inhibitory activities of AP extract and diclofenac on carrageenin-induced hind paw edema in rats are presented in Table 7. In the control group (received distilled water), the edema volume of rat paw was found to increase gradually and reached the maximal increase at the 3rd and the 5th h after carrageenin injection. Likewise diclofenac at the dose of 10 mg/kg body weight, AP extract at the doses of 150, 300 and 600 mg/kg body weight exhibited significant and dose-dependent edema inhibition at all recorded times (R^2 = 0.9781, 0.9845, 0.9304 at the 1st, 3rd and 5th h, respectively). Moreover, the inhibition of AP extract on the edema formation lasted at least 5 h.

3.2.3 Effects of AP extract, diclofenac and prednisolone on 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. Neither AP extract nor diclofenac elicited 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 exhibited a significant inhibitory activity on the edema formation (Table 8).

3.2.4 Effects of AP extract, diclofenac and prednisolone on cotton pellet induced-granuloma formation in rats

3.2.4.1 Effects on granuloma formation

The AP extract and diclofenac significantly reduced the transudative weight but did not produce significant effect on the granuloma formation. However, prednisolone markedly and significantly reduced the transudative weight and inhibited granuloma formation (Table 9).

Table 7 Effects of AP 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	-	0.36 ± 0.04	-	0.67 ± 0.02	-	0.67 ± 0.01	-
Diclofenac	10	0.09 ± 0.01*	75	0.17 ± 0.01*	75	0.25 ± 0.03*	64
AP extract	150	0.24 ± 0.02*	34	0.42 ± 0.03*	38	0.42 ± 0.03*	39
AP extract	300	0.18 ± 0.04*	51	0.31 ± 0.03*	52	0.32 ± 0.03*	53
AP extract	600	0.14 ± 0.03*	61	0.26 ± 0.02*	61	0.29 ± 0.04*	58

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

Significantly different from the control group: * $p < 0.05$

EV = edema volume; % EI = percent edema inhibition

Table 8 Effects of AP extract, diclofenac and prednisolone on AA-induced hind paw edema in rats

Group	Dose (mg/kg)	1 h after AA injection	
		EV (mL)	% EI
Control	-	0.31 ± 0.05	-
Diclofenac	10	0.27 ± 0.04	12
Prednisolone	5	0.12 ± 0.02*	62
AP extract	150	0.26 ± 0.04	15
AP extract	300	0.25 ± 0.04	19
AP extract	600	0.23 ± 0.04	25

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

Significantly different from the control group: * $p < 0.05$

EV = edema volume; % EI = percent edema inhibition

Table 9 Effects of AP extract, diclofenac and prednisolone on granuloma formation and transudation 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	-	392.92 ± 15.53	71.58 ± 2.36	321.33 ± 14.84	2.58 ± 0.12	-
Diclofenac	5	266.92 ± 12.25*	65.25 ± 5.65	201.67 ± 9.48*	2.26 ± 0.28	12
Prednisolone	5	244.25 ± 14.61*	53.42 ± 2.81*	190.83 ± 13.33*	1.67 ± 0.14*	35
AP extract	600	325.83 ± 17.82*	64.33 ± 3.46	261.50 ± 15.78*	2.22 ± 0.17	14

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

Significantly different from the control group: * $p < 0.05$

3.2.4.2 Effects on body weight and thymus weight

Body weight gain and thymus weight of rats in AP extract- and diclofenac-treated groups were not significantly different from those of the control group. On the contrary, prednisolone elicited marked reduction of both parameters when compared to those of the control group (Table 10).

3.2.4.3 Effects on alkaline phosphatase activity

The implantation of cotton pellet in the control rats produced significant increase in serum ALP activity when compared with that of the non-implanted rats in the normal group. This ALP activity was significantly reduced to normal level by diclofenac and prednisolone at the dose of 5 mg/kg/day. However, there was no significant difference in serum ALP activity of rats between AP extract-treated group and the control group (Table 11).

3.2.4.4 Effects on gastric ulcer

As shown in Table 12, the lesions of gastric mucosa of rats in prednisolone-treated group were small and hemorrhagic in nature in 2 of 6 rats, whereas there was no gastric ulceration of rats in the control, diclofenac- and AP extract-treated groups.

3.3 Acute toxicity test

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

Table 10 Effects of AP extract, diclofenac and prednisolone on body weight and thymus weight in cotton pellet-induced granuloma formation in rats

Group	Dose (mg/kg)	Body weight (g)			Dry thymus weight (mg/100 g)
		Initial	Final	Gain	
Control	-	201.67 ± 7.49	262.50 ± 8.34	60.83 ± 2.00	62.41 ± 4.82
Diclofenac	5	193.33 ± 7.15	245.83 ± 4.90	52.50 ± 3.59	55.32 ± 4.66
Prednisolone	5	193.33 ± 4.22	224.17 ± 6.11	30.83 ± 6.11*	32.64 ± 2.64*
AP extract	600	188.33 ± 3.07	243.33 ± 5.58	55.00 ± 5.63	52.90 ± 5.55

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

Significantly different from the control group: * $p < 0.05$

Table 11 Effects of AP 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 alkaline phosphatase activity (U of enz/mg of serum protein x 10^{-4})
Normal	-	190.00 ± 5.70	5.63 ± 0.05	33.71 ± 0.80
Control	-	302.50 ± 18.92	5.32 ± 0.05	56.87 ± 3.43$^{\#}$
Diclofenac	5	166.33 ± 10.55	5.32 ± 0.42	32.77 ± 4.10*
Prednisolone	5	243.83 ± 41.57	5.92 ± 0.20	42.65 ± 8.71*
AP extract	600	273.50 ± 15.30	5.27 ± 0.15	52.26 ± 3.68

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

Significantly different from the normal group: $^{\#}$ $p < 0.05$

Significantly different from the control group: * $p < 0.05$

Table 12 Effects of AP extract, diclofenac and prednisolone on gastric mucosa

Group	Dose (mg/kg)	Ulcer index
Control	-	0
Diclofenac	5	0
Prednisolone	5	1
AP extract	600	0

Results 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