CHAPTER 3

RESULTS

3.1 ANTI-INFLAMMATORY ACTIVITY

3.1.1 Effects of BS extract and diclofenac on EPP-induced ear edema in rats

The inhibitory effects of the topical application of BS extract and diclofenac on EPP-induced ear edema are shown in Table 2. In the control group (received 5% DMSO in acetone), the edema thickness of rat ear increased gradually with time up to 60 min then slightly decreased at 120 min after EPP application. Diclofenac, a COX inhibitor, at the dose of 0.6 mg/ear markedly reduced the ear edema caused by EPP application at all assessment times with the percentages of inhibition of 87, 83, 82 and 79 at 30, 60, 90 and 120 min, respectively. The BS extract at the dose of 1 mg/ear also possessed significant reduction of the EPP-induced edema formation of the rat ear at all evaluation times. The percentages of edema inhibition by BS extrat at 30, 60, 90 and 120 min were 77, 70, 69 and 61, respectively.

3.1.2 Effects of BS extract and diclofenac on carrageenin-induced hind paw edema in rats

The inhibitory activities of oral administration of BS extract and diclofenac on carrageenin-induced hind paw edema in rats are demonstrated in Table 3. In the control group (received distilled water), the edema formation of rat paw increased gradually, peaked at 3 h and was maintained up to 5 h after carrageenin injection. Diclofenac at the dose of 10 mg/kg markedly reduced the edema formation with percentages of inhibition of 77, 76 and 71 at the 1st, 3rd and 5th h, respectively. All doses of BS extract significantly reduced the carrageenin-induced edema formation of the rat paw. At the 3rd h after carrageenin injection, the percentages of the inhibitory effect of the BS extract at the doses of 75, 150 and 300 mg/kg were 45, 55 and 68,

Table 2 Effects of BS extract and diclofenac 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)	% EI	ED (µm)	% EI	ED (µm)	% EI	ED (µm)	% EI
Control	-	117±22	-	157±34	-	197±30	-	172±19	-
Diclofenac	0.6	15±8*	87	27±10*	83	35±13*	82	35±16*	79
BS extract	1	27±8*	77	47±12*	70	61±18*	69	67±20*	61

Test drugs were topically applied to both inner and outer surfaces of the ear.

Control group was applied with 5% DMSO in acetone.

Values are expressed as mean ± S.E.M. (N = 6), Significant difference from control group: * p<0.05

ED = Ear edema, %EI = percent edema inhibition

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

Group	Dose (mg/kg)	1 h		3 h		5 h	
		EV (mL)	% EI	EV (mL)	% EI	EV (mL)	% EI
Control	-	0.35±0.02	-	0.65±0.03	-	0.66±0.04	-
Diclofenac	10	0.08±0.02*	77	0.15±0.07*	76	0.19±0.07*	71
BS extract	75	0.21±0.04*	40	0.36±0.03*	45	0.40±0.04*	39
	150	0.16±0.01*	54	0.29±0.04*	55	0.34±0.07*	48
	300	0.12±0.03*	66	0.21±0.04*	68	0.25±0.06*	62

Test drugs were orally administered 1 h before carrageenin injection.

Control group received distilled water.

Values are expressed as mean ± S.E.M. (N = 6), Significant difference from control group: * p<0.05

EV = Edema volume, %EI = percent edema inhibition

respectively. The antiedematous effect of BS extract seemed to be dose-dependent.

3.1.3 Effects of BS extract, diclofenac and prednisolone on AA-induced hind paw edema in rats

The results of test drugs on AA-induced hind paw edema in rats are illustrated in Table 4. At 1 h after AA injection, the paw edema formation of the control group increased by 0.19±0.03 mL. Diclofenac at the dose of 10 mg/kg did not show any significant inhibitory effect on the edema formation. Similarly, the BS extract at the doses of 75, 150 and 300 mg/kg did not possess significant inhibitory effect on AA-induced hind paw edema. By contrast, prednisolone, a phospholipase A_2 inhibitor, exhibited marked antiedematous effect of 74% at 1 h after AA injection.

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

Group	Dose (mg/kg)	Edema volume (mL)	% El
Control	-	0.19 ± 0.03	-
Diclofenac	10	0.16 ± 0.05	16
Prednisolone	5	0.05 ± 0.06*	74
BS extract	75	0.17 ± 0.03	11
	150	0.15 ± 0.03	21
	300	0.14 ± 0.04	26

Test drugs were orally administered 2 h before AA injection.
Control group received distilled water.
Values are expressed as mean ± S.E.M. (N = 6)
Significant difference from control group: * p<0.05
%EI = percent edema inhibition

3.1.4 Effects of BS extract, diclofenac and prednisolone on the cotton pellet-induced granuloma formation in rats

A. Granuloma formation and transudation

BS extract at the dose of 300 mg/kg and diclofenac at the dose of 2.5 mg/kg slightly but significantly reduced granuloma formation with the percentages of 14 and 15, respectively. Prednisolone at the dose of 5 mg/kg markedly reduced the granuloma formation by 49% (Table 5).

All test drugs significantly reduced the transudative weight in correlation with their inhibitory effects on the granuloma formation. The transudative weight in control group was 261.08 mg. BS extract, diclofenac and prednisolone suppressed the transudative weight to 224.91±39.17, 222.08±27.53 and 172.92±40.26 mg, respectively (Table 5).

B. Alkaline phosphatase activity

The effects of BS extract and both reference drugs on alkaline phosphatase activity are shown in Table 6. Serum ALK activity during cotton pellet implantation in the control group (44.45×10^{-4} U of enzyme/mg of serum protein) was significantly elevated when compared with the normal or non-implanted groups (33.14×10^{-4} U of enzyme/mg of serum protein). The increased serum ALK activity induced by cotton pellet implantation was normalized by 300 mg/kg BS extract, 2.5 mg/kg diclofenac, and 5 mg/kg prednisolone (38.30×10^{-4}, 37.89×10^{-4} and 31.91×10^{-4} U of enzyme/mg of serum protein, respectively).

C. Body weight gain and thymus weight

Body weight gain during the 1st and the 8th day of the experimental period and dry thymus weight of rats implanted with cotton pellets are expressed in Table 7. Body weight gain of the control group was 37.33±7.87 g/wk. The gain of the weight in rats treated with BS extract at the dose of 300 mg/kg (39.33±15.88 g/wk) and diclofenac at the dose of 2.5 mg/kg (30.33±7.63 g/wk) were not different from that of the control group. By contrast, prednisolone at the dose of 5 mg/kg significantly reduced the gain of the body weight (5.67±15.67 g/wk).

Table 5 Effects of BS extract, diclofenac and prednisolone on granuloma weight and transudative weight

Group	Dose (mg/kg)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)	Granuloma inhibition (%)
Control	-	325.25±16.51	64.17±3.29	261.08±14.72	2.20±0.16	-
Diclofenac	2.5	281.33±25.83*	57.58±1.91*	222.08±27.53*	1.88±0.09*	14
Prednisolone	5	216.83±49.08***	42.45±7.99***	172.92±40.26***	1.12±0.39***	49
BS extract	300	284.75±43.74*	57.33±4.85*	224.91±39.17*	1.86±0.25*	15

Test drugs were daily orally administered for 7 days.

Control group received distilled water.

Values are expressed as mean ± S.E.M. (N = 6), Significant difference from control group: * p<0.05, *** p<0.001

Table 6 Effects of BS extract, diclofenac and prednisolone on serum alkaline phosphatase activity

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	-	158.00±12.85	4.82±0.47	33.14±4.79
Control	-	224.33±13.79	5.10±0.40	44.45±5.41†††
Diclofenac	2.5	187.00±9.40	4.97±0.42	37.89±3.93*
Prednisolone	5	161.00±19.17	5.10±0.36	31.91±5.68***
BS extract	300	188.00±20.89	4.93±0.34	38.30±4.61*

Test drugs were daily orally administered for 7 days.

Normal = non-implanted group, Control received distilled water.

Values are expressed as mean ± S.E.M. (N = 6), Significant difference from normal group: ††† p<0.001

Significant difference from control group: * p<0.05, ** p<0.01, *** p<0.001

Table 7 Effects of BS extract, diclofenac and prednisolone on body weight gain and thymus weight

Group	Dose (mg/kg)	Body			Dry thymus weight (mg/100g)
		Initial	Final	Gain	
Control	-	203.33±14.12	240.67±15.16	37.33±7.87	49.09±10.04
Diclofenac	2.5	206.33±7.74	236.67±7.87	30.33±7.63	41.03±1.66
Prednisolone	5	212.00±12.13	217.67±21.67	5.67±15.67***	15.35±2.51***
BS extract	300	195.65±17.64	235.00±16.24	39.33±15.88	40.06±3.06

Test drugs were daily orally administered for 7 days.

Control group received distilled water.

Values are expressed as mean ± S.E.M. (N = 6), Significant difference from control group: *** p<0.001

In the control group, dry thymus weight was 49.09±10.04 mg/100 g body weight. The BS extract and diclofenac did not show any suppressive effect on thymus weight (40.06±3.06 and 41.03±1.66 mg/100 g body weight, respectively), whereas prednisolone markedly reduced thymus weight to 15.35±2.51 mg/100 g body weight.

3.2 ANALGESIC ACTIVITY

3.2.1 Effects of BS extract and diclofenac on acetic acid-induced writhing response in mice

The results of the acetic acid-induced writhing response in mice are shown in Table 8. In the control group, intraperitoneal injection of 0.75% acetic acid produced 29.33±8.04 writhes during the period of 15 min, beginning of 5 min after acetic acid injection. Diclofenac at the dose of 10 mg/kg exhibited significant inhibition of the number of writhes with the percentage of 78. BS extract at the dose of 75, 150 and 300 mg/kg significantly inhibited writhing response by 35%, 42% and 91%, respectively.

3.2.2 Effects of BS extract, diclofenac and codeine on the tail-flick test in rats

The results in Table 9 demonstrate analgesic effect of BS extract, diclofenac and codeine on the tail-flick response in rats. The inhibitory effect of test drugs on algesia induced by infrared lamp application to the tail of rats was investigated using the rats with normal reaction time to flick the tail within 2-4 sec. Codeine, an opioid drug, at the dose of 75 mg/kg produced analgesic activity with the percentages of 61. BS extract at the dose of 300 mg/kg and diclofenac at the dose of 10 mg/kg similarly, did not show any activity on the tail-flick response.

Table 8 Effects of BS extract and diclofenac on acetic acid-induced writhing response in mice

Group	Dose (mg/kg)	No. of writhes	Inhibition of writhing response (%)
Control	-	29.33±8.04	-
Diclofenac	10	6.33±4.13*	78
BS extract	75	19.17±10.91*	35
	150	17.00±7.46*	42
	300	2.50±3.99*	91

Test drugs were orally administered 1 h before acetic acid injection.

Control group received distilled water.

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

Significant difference from control group: * p<0.05

Table 9 Effects of BS extract, diclofenac and codeine on tail-flick test in rats

Group	Dose (mg/kg)	T_c (s)	T_t (s)	% inhibition
Control	-	2.28±0.17	2.58±0.22	-
Diclofenac	10	2.45±0.45	2.88±0.55	6
Codeine	75	2.33±0.22	7.00±0.90*	61
BS extract	300	2.31±0.26	2.75±0.37	6

Test drugs were orally administered 1 h before re-exposure to radiant heat.

Control group received distilled water.

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

Significant difference from control group: * p<0.05

T_c = control reaction time, T_t = reaction time after receiving test drugs

3.3 ANTIPYRETIC ACTIVITY

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

The effects of BS extract and diclofenac on yeast-induced hyperthermia in rats is shown in Table 10. Rectal temperature of rats received diclofenac at the dose of 10 mg/kg was reduced from 38.7±0.4 to 38.1±0.2, 37.5±0.4, 37.5±0.4 and 37.4±0.4 ^{o}C at 30, 60, 90 and 120 min after drug administration, respectively. The BS extract at the dose of 150 mg/kg slightly reduced pyrexia only at 120 min after treatment, whereas the extract at the dose of 300 mg/kg significantly reduced pyrexia in rats at all assessment times.

Table 10 Effects of BS extract and diclofenac on yeast-induced hyperthermia in rats

Group	Dose (mg/kg)	Rectal temperature (^{o}C)					
		Before yeast injection	18 h after yeast injection	Time after drug administration			
				30 min	60 min	90 min	120 min
Control	-	37.6±0.3	38.8±0.3	38.8±0.5	38.7±0.5	38.7±0.5	38.7±0.6
Diclofenac	10	37.5±0.5	38.7±0.4	38.1±0.1**	37.7±0.4***	37.4±0.5***	37.3±0.5***
BS extract	150	37.6±0.3	38.9±0.5	38.7±0.4	38.4±0.5	38.2±0.5	38.0±0.5*
	300	37.6±0.4	38.7±0.4	38.4±0.4*	38.1±0.2**	37.8±0.4**	37.7±0.2**

Test drugs were orally administered after recording the rectal temperature at 18 h after yeast injection.

Control group received distilled water.

Values are expressed as mean ± S.E.M. (N = 6), Significant difference from control group: * p<0.05, ** p<0.01, *** p<0.001