RESULTS

ANTI-INFLAMMATORY ACTIVITY

1. Effects of DVHE and aspirin on carrageenin-induced hind paw edema in rats

The inhibitory activity on carrageenin-induced rat hind paw edema caused by an oral administration of DVHE and aspirin at various times after carrageenin injection is shown in Table 1. Aspirin, a cyclooxygenase inhibitor, at the dose of 300 mg/kg exhibited significant edema inhibitory activity. DVHE at doses of 75, 150 and 300 mg/kg possessed moderate inhibitory effect on carrageenin-induced paw edema at all assessment times. The percent inhibition on the edema formation of DVHE was gradually increased as the doses increased. The anti-inflammatory effect of DVHE on the paw edema formation at the dose of 300 mg/kg was less effective than that of aspirin at the same dose. The percent edema inhibition produced by the dose of 300 mg/kg of DVHE on carrageenin-induced edema formation of the rat paw were 46, 41 and 37 whereas those produced by aspirin were 58, 52 and 46 at the 1st, 3rd and 5th h, respectively, after carrageenin injection. Both DVHE and aspirin still possessed the inhibitory effect on the edema formation 5 hours after drug treatment, although this effect was slightly less than that at the 1st and 3rd h.

2. Effects of DVHE, aspirin and prednisolone on carrageenin-induced pleurisy in rats

2.1 Pleural exudate volume

Table 2 demonstrates the anti-exudate formation effect of DVHE, aspirin and prednisolone obtained from rat pleurisy model. The pleural exudate induced by an intrapleural injection of carrageenin was harvested either 3 or 6 hours thereafter. The results obtained revealed that the three drugs tested exhibited

Table 1. Inhibitory effect of DVHE and aspirin 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	-	0.26 ± 0.02	-	0.58 ± 0.02	-	0.71 ± 0.01	-
Aspirin	300	0.11 ± 0.02*	58	0.28 ± 0.02*	52	0.38 ± 0.02*	46
DVHE	75	0.18 ± 0.01*	31	0.44 ± 0.01*	24	0.56 ± 0.02*	21
	150	0.15 ± 0.01*	42	0.40 ± 0.02*	31	0.52 ± 0.01*	27
	300	0.14 ± 0.02*	46	0.34 ± 0.01*	41	0.45 ± 0.01*	37

Test drugs were orally administered 1 h before carrageenin injection

Control = received 5% Tween 80

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

Significantly different from control: * $p < 0.05$

EV = edema volume; EI = edema inhibition

Table 2. Inhibitory effect of DVHE, aspirin and prednisolone on pleural exudate volume of carrageenin-induced pleurisy in rats

Group	Dose (mg/kg)	Time after carrageenin injection			
		3 h		6 h	
		ExV (ml)	ExVI (%)	ExV (ml)	ExVI (%)
Control	-	0.43 ± 0.03	-	1.41 ± 0.08	-
Aspirin	300	0.09 ± 0.02*	79	0.37 ± 0.02*	74
Prednisolone	5	0.24 ± 0.02*	44	0.49 ± 0.06*	65
DVHE	300	0.23 ± 0.01*	47	0.83 ± 0.04*	41

Test drugs were orally administered 30 minutes before carrageenin injection

Control = received 5% Tween 80

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

Significantly different from control: * $p < 0.05$

ExV = exudate volume; ExVI = exudate volume inhibition

significantly inhibitory activity on the formation of the pleural exudate at both times of exudate harvesting. Three hours after carrageenin injection, DVHE at a dose of 300 mg/kg showed less anti-exudative activity (47%) than aspirin at the same dose level (79%) but anyhow its effect was similar to that of prednisolone (the steroidal anti-inflammatory drug) at a dose of 5 mg/kg (44%). The inhibitory activity of aspirin and DVHE on the exudative formation at the 6th hour was slightly less than that found at the 3rd hour. On the contrary, prednisolone exhibited greater anti-exudative activity, when pleural exudate was harvested 6 hours after carrageenin injection. Corresponding to the inhibitory activity on pleural exudating fraction at the 3rd hours, DVHE also showed less anti-exudative effect than aspirin when assessed 6 hours after intrapleural injection of carrageenin. The inhibitory effect of aspirin, prednisolone and DVHE was found to be 74%, 65% and 41%, respectively.

2.2 Total leukocytes count in the pleural exudate

The inhibitory effects of DVHE, aspirin and prednisolone on the number of the total leukocytes were determined at 3 and 6 h after carrageenin injection. As shown in Table 3, the results obtained revealed that aspirin and prednisolone exhibited significantly inhibitory activity on the leukocytes accumulation in the pleural exudate at both times of exudate harvesting. In contrast, DVHE exhibited a non significantly inhibitory activity on the leukocytes accumulation in the pleural exudate at both times of exudate harvesting. The pattern of inhibitory effect of the three drugs tested on the leukocyte accumulation in the pleural exudate was found to be correlated to the exudate formation. The intensity of inhibition of aspirin and DVHE on leukocyte number at the 3rd hour was greater than that at the 6th hour, whereas prednisolone showed less activity at the 3rd hour than at the 6th hour. The percent inhibition on the leukocytic mobilization of aspirin (300 mg/kg), prednisolone (5 mg/kg) and DVHE (300 mg/kg) was found to be 40, 34 and 15 at the 3rd hour and 30, 38 and 4 at the 6th hour, respectively.

Table 3. Inhibitory effect of DVHE, aspirin and prednisolone on total leukocyte number of carrageenin-induced pleurisy in rats

Group	Dose (mg/kg)	Time after carrageenin injection			
		3 h		6 h	
		TLN ($x10^7$ cells/ml)	TLNI (%)	TLN ($x10^7$ cells/ml)	TLNI (%)
Control	-	4.51 ± 0.31	-	14.04 ± 0.33	-
Aspirin	300	2.72 ± 0.16*	40	9.81 ± 0.33*	30
Prednisolone	5	2.99 ± 0.25*	34	8.69 ± 0.28*	38
DVHE	300	3.85 ± 0.59	15	13.51 ± 0.34	4

Test drugs were orally administered 30 minutes before carrageenin injection

Control = received 5% Tween 80

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

Significantly different from control: * $p < 0.05$

TLN = total leukocyte number; TLNI = total leukocyte number inhibition

3. Effects of DVHE, aspirin and prednisolone on the cotton pellet-induced granuloma formation in rats

The inhibitory effects of DVHE and reference drugs 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 values of the inhibitory action of DVHE and reference drugs against granuloma formation induced by cotton pellet implantation are shown in Table 4. It was found that the steroidal anti-inflammatory drug, prednisolone, at the dose of 5 mg/kg exhibited significantly inhibitory effect on the granuloma formation whereas the nonsteroidal anti-inflammatory drug, aspirin, and DVHE, at the dose of 300 mg/kg elicited a nonsignificant inhibition on the granuloma formation. The granuloma inhibitory effect of prednisolone was found to be 39% whereas that effect of aspirin and DVHE was found to be 18% and 10%, respectively. In control group the transudative weight was found to be 263.19 mg. It was found that DVHE and aspirin did not significantly reduced the transudative weight. The transudative weight of DVHE and aspirin treated groups was found to be 235.39 and 233.35 mg, respectively. Only prednisolone significantly reduced the weight of transudate to 189.65 mg.

4. Effects of DVHE, aspirin and prednisolone on the alkaline phosphatase activity in serum

The effects of DVHE, aspirin and prednisolone on the alkaline phosphatase activity in serum of rats implanted with cotton pellets are shown in Table 5. Significant elevated alkaline phosphatase activity in serum of rats in control group was observed (50.37×10^{-4} U of enz./mg of serum protein) when compared with that of normal or non-implanted rats (34.15×10^{-4} U of enz./mg of serum protein). It was found that the increase in serum alkaline phosphatase activity caused by cotton pellet implantation was reduced to normal level by DVHE (33.21×10^{-4} U of enz./mg of serum protein) and aspirin at the dose of 300 mg/kg (31.94×10^{-4} U of

Table 4. Effects of DVHE, aspirin and prednisolone on cotton pellet-induced granuloma in rats

Group	Dose (mg/kg)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma formation (mg/mg cotton)	GI (%)
Control	-	324.16 ± 21.07	60.97 ± 3.43	263.19 ± 18.68	2.05 ± 0.17	-
Aspirin	300	289.18 ± 15.97	53.79 ± 1.77	235.39 ± 14.67	1.69 ± 0.09	18
Prednisolone	5	234.63 ± 23.25*	44.98 ± 3.16*	189.65 ± 20.55*	1.25 ± 0.16*	39
DVHE	300	290.10 ± 18.72	56.75 ± 2.42	233.35 ± 16.53	1.84 ± 0.12	10

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

Significantly different from control: * $p < 0.05$

Control = received 5% Tween 80

GI = granuloma inhibition

Table 5. Effects of DVHE, aspirin and prednisolone on the alkaline phosphatase activity in serum

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})
Control	-	291.50 ± 31.85	5.82 ± 0.38	50.37 ± 5.37[a]
Aspirin	300	176.43 ± 8.58	5.56 ± 0.20	31.94 ± 1.84[b]
Prednisolone	5	188.50 ± 23.38	5.97 ± 0.27	31.37 ± 3.01[b]
DVHE	300	181.83 ± 21.86	5.57 ± 0.33	33.21 ± 4.83[b]

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

a = significantly different from normal: $p < 0.05$; b = significantly different from control: $p < 0.05$

Control = implanted group, received 5% Tween 80

Normal = non-implanted group (Alkaline phosphatase = 209.17 ± 17.55; Total protein = 6.13 ± 0.30; Serum alkaline phosphatase activity = 34.15 ± 2.49)

enz./mg of serum protein) as well as by prednisolone at the dose of 5 mg/kg (31.37 x 10^{-4} U of enz./mg of serum protein). The inhibitory effect of DVHE at the dose of 300 mg/kg on the elevated alkaline phosphatase activity in serum of cotton pellet-implanted rats was found to be comparable to those of reference drugs, aspirin at the dose of 300 mg/kg and prednisolone at the dose of 5 mg/kg.

5. Effects of DVHE, aspirin and prednisolone on the thymus weight and the body weight gain

Results demonstrated in Table 6 show the body weight gain during the first and the last day of experimental period and the dry weight of thymus of the rats implanted with cotton pellets. In control group the body weight gain in one week was 38.00 g. Aspirin and DVHE at the dose of 300 mg/kg did not affect the body weight gain of animals. The gain of the weight in aspirin and DVHE treated group were 36.51 and 38.33 g, respectively, which were not significantly different from that of control group. On the contrary, prednisolone, at the dose of 5 mg/kg significantly reduced the gain of the body weight to 8.67 g. Dry thymus weight of rats in control group was 45.10 mg/100 g body weight. Both DVHE and aspirin did not showed any suppressive effect on the thymus weight (46.07 and 43.39 mg/100 g body weight, respectively) of the rats when compared with control group, whereas prednisolone significantly reduced the thymus weight of the rats to 27.59 mg/100 g body weight.

ULCEROGENIC ACTIVITY

1. Effects of DVHE and aspirin on gastric mucosa in rats

Results demonstrated in Table 7 show the effect of DVHE in comparison with aspirin on gastric mucosa of rats on the eighth day after the daily oral administration of test drugs for 7 days. It was found that only the nonsteroidal anti-inflammatory drug, aspirin, exhibited significant gastric ulceration with ulcer

Table 6. Effects of DVHE, aspirin and prednisolone on the thymus weight and the body weight gain

Group	Dose (mg/kg)	Body weight (g)			Dry thymus weight (mg/100 g)
		Initial	Final	Gain	
Control	-	177.67 ± 1.40	215.67 ± 4.94	38.00 ± 4.76	45.10 ± 5.44
Aspirin	300	185.14 ± 3.95	221.71 ± 3.92	36.51 ± 3.32	43.39 ± 2.86
Prednisolone	5	174.33 ± 2.50	183.00 ± 5.60	8.67 ± 4.28*	27.59 ± 2.50*
DVHE	300	179.33 ± 2.72	217.67 ± 5.23	38.33 ± 3.98	46.07 ± 3.36

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

Significantly different from control: * $p < 0.05$

Control = received 5% Tween 80

index of 4.83 mm. On the contrary, DVHE did not affect the gastric mucosa of the rats when compared with those in the control group (0 mm).

Table 7. Effects of DVHE and aspirin on gastric mucosa in rats

Group	Ulcer index (mm)
Control	0
Aspirin 300 mg/kg	4.83 ± 0.31*
DVHE 300 mg/kg	0

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

Significantly different from control: * $p < 0.05$

Control = received 5% Tween 80

ANTI-ULCEROGENIC ACTIVITY

1. Indomethacin-induced gastric lesions in rats

Table 8 demonstrates the data obtained when DVHE and misoprostol (the reference drug) were tested against indomethacin-induced gastric lesions. Intraperitoneal administration of indomethacin caused gastric ulceration with ulcer index of 11.00 mm (control group). DVHE was found to exhibit only slight anti-ulcer activity, without causing statistical significant reduction of ulcer formation induced by indomethacin. Ulcer index observed with DVHE administration at the dose of 300 mg/kg was 8.83 mm with the percent inhibition of 20%. Misoprostol at the dose of 100 µg/kg showed anti-ulcer activity with the ulcer index of 1.00 mm, and percent inhibition of 91%.

Table 8. Effects of DVHE and misoprostol on indomethacin-induced gastric lesions in rats

Group	Ulcer index (mm)	Inhibition (%)
Control	11.00 ± 0.73	-
Misoprostol 100 μg/kg	1.00 ± 0.22*	91
DVHE 300 mg/kg	8.83 ± 0.70	20

Drugs were orally administered 1 h before induce gastric lesion

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

Significantly different from control: * $p < 0.05$

Control = received 5% Tween 80

2. Pylorus ligation

The effects of DVHE and cimetidine on gastric volume, gastric secretory rate and total acidity were investigated in this model, and the results obtained are shown in Table 9. The gastric volume (8.87 ml), gastric secretory rate (0.92 ml/100 g body weight/h) and total acidity (135.71 mEq/l) were observed in the control group. Cimetidine (H_2 antagonist) at the dose of 100 mg/kg showed an anti-secretory effect, causing a significant decrease of gastric volume (3.47 ml), gastric secretory rate (0.34 ml/100 g body weight/h) and total acidity (54.62 mEq/l). Percent inhibitions of cimetidine on gastric volume, gastric secretory rate and total acidity were 61, 63 and 60, respectively. The gastric volume (9.60 ml), gastric secretory rate (0.94 ml/100 g body weight/h) and total acidity (126.47 mEq/l) of groups pretreated with DVHE at the doses of 300 mg/kg did not show any statistical difference from those of the control group.

Table 9. Effects of DVHE and cimetidine on pylorus-ligated rats

9.1 Gastric volume

Group		Gastric volume (ml)	Inhibition (%)
Control		8.87 ± 0.64	-
Cimetidine	100 mg/kg	3.47 ± 0.31*	61
DVHE	300 mg/kg	9.60 ± 0.52	-

9.2 Gastric secretory rate

Group		Gastric secretory rate (ml/100 g/h)	Inhibition (%)
Control		0.92 ± 0.08	-
Cimetidine	100 mg/kg	0.34 ± 0.04*	63
DVHE	300 mg/kg	0.94 ± 0.05	-

9.3 Total acidity

Group		Total acidity (mEq/l)	Inhibition (%)
Control		135.71 ± 8.28	-
Cimetidine	100 mg/kg	54.62 ± 6.33*	60
DVHE	300 mg/kg	126.47 ± 5.63	-

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

Significantly different from control: * $p < 0.05$

Control = received 5% Tween 80