RESULTS

ANTI-INFLAMMATORY TEST

1. Effect of GS extract and phenylbutazone on EPP-induced ear edema in rats

The inhibitory effect produced by the topical administration of GS extract on EPP-induced rat ear edema was assessed at 15, 30, 60 and 120 min after EPP application as shown in Table 1.

Phenylbutazone, the nonsteroidal anti-inflammatory drug, at the dose of 1 mg/ear, exhibited significant inhibitory activity on the edema formation at all determination times. It produced marked antiedema activity of 73%, 60%, 47% and 42% at 15, 30, 60 and 120 min, respectively. At doses of 1, 2 and 3 mg/ear, GS extract also possessed profound inhibitory effect on EPP-induced rat ear edema at all assessment times. The percent inhibition on the edema formation was gradually increased as the dose increased. The results showed that a marked effect of GS extract was obtained with the highest dose used in this test i.e. 3 mg/ear. This dose showed significant inhibitory effect on edema formation of 55%, 57%, 50% and 48% at 15, 30, 60 and 120 min, respectively, after topical application of EPP.

2. Effect of GS extract, phenlbutazone and phenidone on arachidonic acid-induced ear edema in rats

Results obtained from the rat ear edema induced by AA are demonstrated in Table 2. Phenylbutazone, a cyclooxygenase inhibitor, at the dose of 1 mg/ear did not show any inhibitory effect on AA-induced edema. In contrast, phenidone, a dual inhibitor of AA metabolism, exhibited marked inhibitory activity on the edema formation of 69%, 52%, 47% and 48% when assessment was done 15, 30, 60 and 120 min after AA application. At doses

Table 1. Inhibitory effect of GS extract and phenylbutazone on EPP-induced ear edema in rats.

Group	Dose (mg /ear)	Time after topical application of EPP							
		15 min		30 min		1h		2 h	
		ED (um)	EDI (%)	ED (um)	EDI (%)	ED (um)	EDI (%)	ED (um)	EDI (%)
Control	-	170.00 ± 6.83	-	300.00 ± 13.83	-	306.67 ± 11.16	-	220.00 ± 11.55	-
Phenylbutazone	1	46.67 ± 4.23*	73	120.00 ± 11.55*	60	163.33 ± 8.03*	47	126.67 ± 12.29*	42
GS extract	1	116.67 ± 12.02*	31	160.00 ± 10.33*	47	213.33 ± 12.44*	30	130.00 ± 19.15*	41
	2	93.33 ± 4.22*	45	133.33 ± 8.43*	56	186.67 ± 4.22*	39	120.00 ± 19.32*	45
	3	76.67 ± 3.33*	55	130.00 ± 11.25*	57	153.33 ± 8.43*	50	113.33 ± 16.06*	48

Test drugs were applied topically to both inner and outer surfaces of the ear. Control = received ethanol only.

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

Table 2. Inhibitory effect of GS extract, phenylbutazone and phenidone on AA-induced ear edema in rats.

Group	Dose (mg /ear)	Time after topical application of AA							
		15 min		30 min		1h		2 h	
		ED (um)	EDI (%)	ED (um)	EDI (%)	ED (um)	EDI (%)	ED (um)	EDI (%)
Control	-	47.50 ± 3.66	-	77.50 ± 5.90	-	85.00 ± 9.10	-	62.50 ± 4.53	-
Phenylbutazone	1	43.33 ± 6.15	9	63.33 ± 6.15	18	73.33 ± 6.61	14	50.00 ± 4.47	20
Phenidone	1	15.00 ± 5.00*	69	37.50 ± 4.53*	52	45.00 ± 3.27*	47	32.50 ± 3.66*	48
GS extract	1	32.50 ± 3.36*	32	47.50 ± 3.66*	39	55.00 ± 5.00*	35	55.00 ± 5.00	12
	2	30.00 ± 5.35*	37	40.00 ± 6.55*	48	50.00 ± 7.56*	41	42.50 ± 4.53*	32
	3	22.50 ± 4.53*	53	35.00 ± 5.00*	55	42.50 ± 5.90*	50	35.00 ± 5.00*	44

Test drugs were applied topically to both inner and outer surfaces of the ear. Control = received ethanol only.

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

of 1, 2 and 3 mg/ear, GS extract possessed profound inhibitory effect on AA-induced rat ear edema at all assessment times (except at the dose 1 mg/ear at 120 min after AA application). The percent inhibition on the edema formation was gradually increased as the dose increased. The result showed that a marked effect of GS extract was obtained with the highest dose used in this test i.e. 3 mg/ear. This dose showed significant inhibitory effect on edema formation of 53%, 55%, 50% and 44% at 15, 30, 60 and 120 min, respectively, after topical application of AA.

3. Effect of GS extract 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 GS extract and aspirin at various times after carrageenin injection is shown in Table 3.

Aspirin, a cyclooxygenase inhibitor, at the dose of 150 mg/kg exhibited significant edema inhibitory activity of 40%, 49% and 46% at the 1^{st}, 3^{rd} and 5^{th} h, respectively. GS extract at doses of 75, 100, 150 and 300 mg/kg possessed profound inhibitory effect on carrageenin-induced paw edema at all assessment times. The inhibitory effect of GS extract, at doses of 150 and 300 mg/kg on the paw edema formation was similar at all determination times. The anti-inflammatory effect of GS extract on the paw edema formation at the dose of 150 mg/kg was more effective than that of aspirin at the same dose. The percent edema inhibition produced by the dose of 150 mg/kg of GS extract on carrageenin-induced edema formation of the rat paw were 64, 80 and 78 whereas those produced by aspirin were 40, 49 and 46 at the 1^{st}, 3^{rd} and 5^{th} h, respectively, after carrageenin injection. Both GS extract and aspirin still possessed the inhibitory effect on

Table 3. Inhibitory effect of GS extract and aspirin on carrageenin-induced 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	-	0.25 ± 0.04	-	0.75 ± 0.10	-	0.69 ± 0.05	-
Aspirin	150	0.15 ± 0.01*	40	0.38 ± 0.01*	49	0.37 ± 0.01*	46
GS extract	75	0.17 ± 0.02*	32	0.47 ± 0.01*	37	0.46 ± 0.07*	33
	100	0.15 + 0.02*	40	0.39 + 0.02*	48	0.37 + 0.02*	46
	150	0.09 ± 0.01*	64	0.15 ± 0.01*	80	0.15 ± 0.01*	78
	300	0.10 ± 0.01*	60	0.15 ± 0.10*	80	0.15 ± 0.01*	78

Test drugs were orally administered 1 h before carrageenin injection. Control = received 5% ethanol in 30% acacia. Values are expressed as mean ± S.E.M. ($N = 6$). Significantly different from control: * $P < 0.05$.

the edema formation 5 h after drug treatment, although this effect was slightly less than that at the 3rd h.

4. Effect of GS extract, indomethacin and prednisolone on the cotton pellet-induced granuloma formation

The inhibitory effect of GS extract and reference drugs on the cotton pellet-induced granuloma formation in rats was examined on the eighth day after the daily oral administration of test drugs for 7 days. The values of the inhibitory action of GS extract and reference drugs against granuloma formation induced by cotton pellet implantation are shown in Table 4.1. It was found that the nonsteroidal anti-inflammatory drug, indomethacin, and the steriodal anti-inflammatory drug, prednisolone, at the dose of 5 mg/kg exhibited significantly inhibitory effect on the granuloma formation whereas GS extract, at the dose of 150 mg/kg elicited nonsignificant inhibition on the granuloma formation. The granuloma inhibitory effect of prednisolone and indomethacin was found to be 46% and 20 %, respectively. In control group the transudative weight was found to be 342.64 mg. Only prednisolone and indomethacin, significantly reduced the weight of the transudate to 249.30 and 267.30 mg, respectively.

Results demonstrated in Table 4.2 show the body weight gain during the first and the last day of experimental period and the dry weight of thymuses of the rats implanted with cotton pellets. In control group the body weight gain in one week was 61.20 $\pm$ 1.2 g. Indomethacin (5 mg/kg) and GS extract, did not affect the body weight gain of animals. The gain of the weight in indomethacin and GS extract treated groups were 57.80 $\pm$ 1.50 and 58.17 $\pm$ 1.38 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 39.60 $\pm$ 0.81 g. Dry

Table 4. Effects of GS extract, indomethacin and prednisolone on cotton pellet-induced granuloma in rats.

4.1 Granuloma formation and transudation.

Group	Dose (mg/kg)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)	GI (%)
Control	-	414.76 ± 0.56	72.12 ± 0.74	342.64 ± 0.98	2.61 ± 0.04	-
Indomethacin	5	328.80 ± 3.31*	61.50 ± 1.11*	267.30 ± 2.30*	2.07 ± 0.06*	20
Prednisolone	5	297.44 ± 1.65*	48.14 ± 0.27*	249.30 ± 1.46*	1.42 ± 0.01*	46
GS extract	150	406.70 ± 3.01	68.78 ± 0.43	337.92 ± 2.61	2.44 ± 0.02	6

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

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

GI = granuloma inhibition. Control = received 5% ethanol in 30% acacia.

Table 4. (cont.)

4.2 Body weight and thymus weight.

Group	Dose (mg/kg)	Body weight (g)			Dry thymus weight (mg/100 g)
		Initial	Final	Gain	
Control	-	192.60 ± 21.30	253.80 ± 21.70	61.20 ± 1.20	49.92 ± 2.79
Prednisolone	5	181.80 ± 10.27	221.40 ± 10.96	39.60 ± 0.81*	33.82 ± 2.57*
Indomethacin	5	188.00 ± 9.70	245.80 ± 10.44	57.80 ± 1.50	48.15 ± 1.98
GS extract	150	177.67 ± 3.12	235.83 ± 3.01	58.17 ± 1.38	50.08 ± 2.10

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

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

Control = received 5% ethanol in 30% acacia.

thymus weight of rats in control group was 49.92 ± 2.79 mg/100 g body weight. Both GS extract and indomethacin did not showed any suppressive effect on the thymus weight (50.08 ± 2.10 and 48.15 ± 1.98 mg/100 g body weight, respectively) of the rats when compared with control group, whereas prednisolone significantly reduced the thymus weight of the animals to 33.82 ± 2.57 mg/100 g body weight.

The effects of test drugs on alkaline phosphatase activity in rats implanted with cotton pellets are shown in Table 4.3. Significant elevated alkaline phosphatase level in the serum of rats in control group was observed (49.83×10^{-4} U of enz./mg of serum protein) when compared with that of normal or non-implanted rats (42.36×10^{-4} U of enz./mg of serum protein). The increase in serum alkaline phosphatase caused by cotton pellet implantation was reduced to normal level by GS extract at the dose of 150 mg/kg (38.14×10^{-4} U of enz./mg of serum protein) as well as by both indomethacin at the dose of 5 mg/kg (38.64×10^{-4} U of enz./mg of serum protein) and prednisolone at the dose of 5 mg/kg (38.67×10^{-4} U of enz./mg of serum protein). The inhibitory effect of GS extract at a dose of 150 mg/kg on the elevated alkaline phosphatase in the serum of cotton pellet-implanted rats (38.14×10^{-4} U of enz./mg of serum protein) was found to be comparable to those of reference drugs, indomethacin and prednisolone, at the dose of 5 mg/kg.

5. Effect of GS extract, morphine and aspirin on acetic acid-induced writhing response in mice

As shown in Table 5 morphine at a dose of 10 mg/kg completely inhibited the writhing response. Aspirin at doses of 25.0, 37.5 and 50.0 mg/kg also exerted significant inhibition on the number of writhes induced in mice by acetic acid with the percentage of inhibition of 36, 72 and 84

Table 4. (cont.)

4.3 Alkaline phosphatase activity in the serum.

Group	Dose (mg/kg)	Alkaline phosphatase (units/l)	Total protein (g/dl)	Serum alkaline phoshatase activity (U of enz./mg of serum protein x 10^{-4})
Normal	-	233 ± 84	5.50 ± 0.50	42.36 ± 1.10
Control	-	306 ± 31	6.12 ± 0.21	49.83 ± 4.32[a]
Prednisolone	5	236 ± 8	6.10 ± 0.06	38.67 ± 0.88[b]
Indomethacin	5	214 ±16	5.53 ± 0.29	38.64 ± 0.95[b]
GS extract	150	209 ±12	5.48 ± 0.14	38.14 ± 2.15[b]

Values are expressed as mean ± S.E.M. ($N = 5$).

a = significant from normal: $P < 0.05$. b = significant from control: $P < 0.05$.

Normal = non-implanted group; Control = implanted group, received 5% ethanol in 30% acacia.

respectively. The inhibitory effect of aspirin was found to be dose-related (r = 0.9792). The effective inhibitory dose at 50% (ED_{50}) of aspirin determined from its dose-response curve (Figure 5) was found to be 29.55 mg/kg. GS extract was found to possess profound inhibitory activity on writhing response. At doses of 25.0, 37.5 and 50.0 mg/kg, GS extract showed reduction of writhes with the percentage of 24, 49 and 65, respectively. The inhibitory effect on the writhing response of GS extract was dose-related with the r value of 0.9988. The ED_{50} value of GS extract determined from its dose-response curve (Figure 7) was found to be 38.53 mg/kg.

Table 5. Inhibitory activity of GS extract and aspirin on writhing response in mice.

Group	Dose (mg/kg)	No. of writhes	Decrease of writhing response (%)
Control	-	26.7 ± 4.0	-
Morphine	10.0	0.00*	100
Aspirin	25.0	17.0 ± 7.4*	36
	37.5	7.5 ± 1.7*	72
	50.0	4.2 ± 1.7*	84
GS extract	25.0	20.2 ± 3.2*	24
	37.5	13.6 ± 1.3*	49
	50.0	9.4 ± 0.4*	65

Drugs were intraperitoneally administered 30 min before acetic acid injection.

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

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

Control = received 5% ethanol in 30% acacia

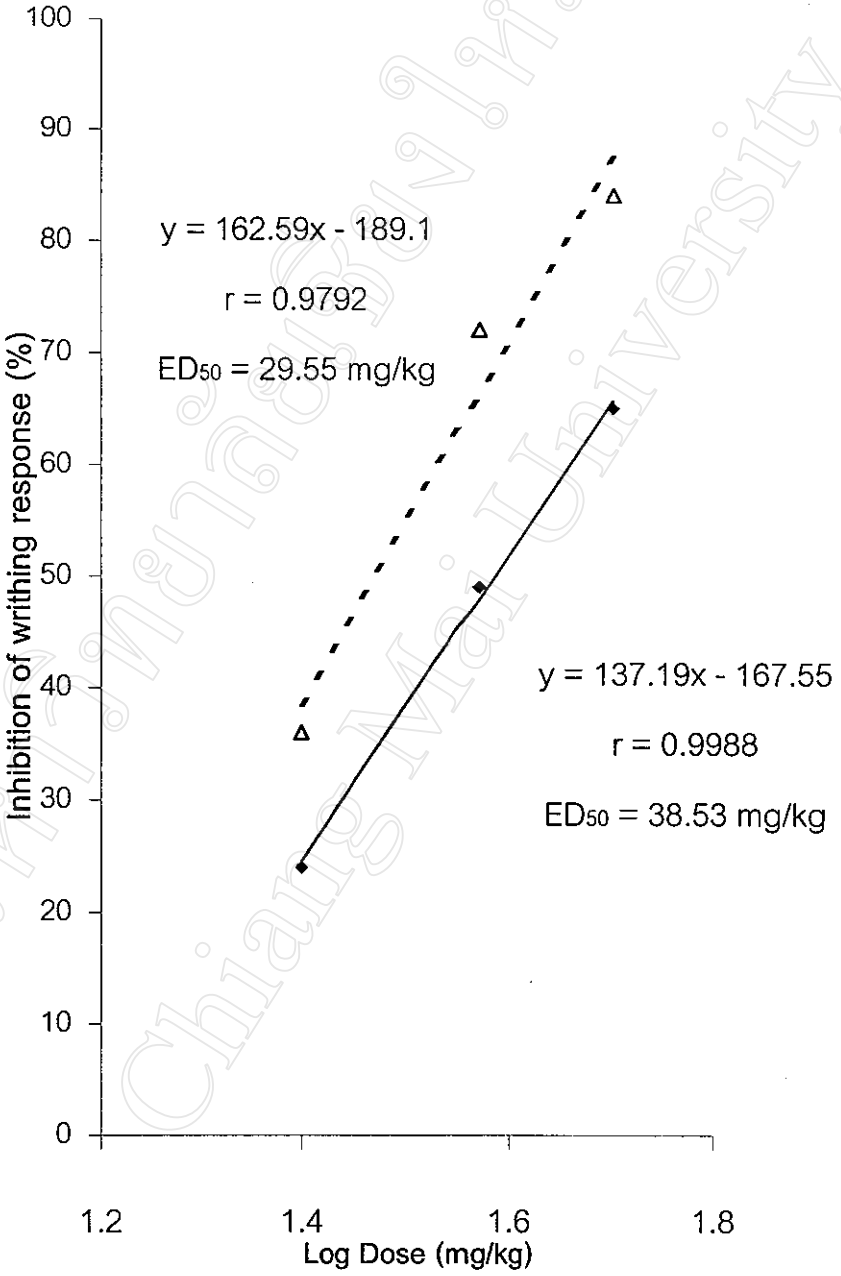

Figure 7. Log dose-response regression line of aspirin (- △ -) and GS extract (—◆—) on writhing response.

ANTI-ULCEROGENIC TEST

1. Pylorus ligation-induced gastric lesions in rats

The effects of GS extract and cimetidine on gastric secretory rate and total acidity were investigated in this model, and the results obtained are shown in Table 6. The gastric volume of 6.42 ml, gastric secretion rate of 0.76 ml/100 g/h and total acidity of 195.09 mEq/100 g were observed in the control group. The gastric volume, gastric secretion rate and total acidity of groups pretreated with GS extract at the doses of 75,150 and 300 mg/kg did not show any statistical difference from that of the control group, whereas cimetidine showed an anti-secretory effect, causing a significant decrease of gastric volume (3.37 ml), gastric secretion rate (0.36 ml/100 g/h) and total acidity (57.83 mEq/100 g).

2. Indomethacin-induced gastric lesions in rats

Table 7 demonstrates the data obtained with GS extract and misoprostrol (the reference drug). Intraperitoneal administration of indomethacin caused gastric ulceration with an ulcer index of 2.50 (control group). GS extract was found to exhibit anti-ulcer activity, causing statistical reduction of ulcer formation induced by indomethacin. Ulcer index observed with GS extract administered at the doses of 75, 150 and 300 mg/kg were 1.80, 1.71 and 1.17, respectively. Percent inhibition of ulcer formation increased with the increasing dose of GS extract which was found to be 28, 31 and 53 with the dose of 75, 150 and 300 mg/kg, respectively. Misoprostol showed anti-ulcer activity with the ulcer index of 0.50 and percent inhibition of 80.

Table 6. Effect of GS extract on gastric acid secretion in pylorus-ligated rats.

Group		Gastric volume (ml)	Gastric secretion rate (ml/100 g/h)	Total acidity (mEq/100 g)
Control		6.42 ± 1.39	0.76 ± 0.10	195.09 ± 8.16
Cimetidine	100 mg/kg	3.37 ± 0.62*	0.36 ± 0.07*	57.83 ± 14.83*
GS extract	75 mg/kg	7.28 ± 0.87	0.71 ± 0.10	135.36 ± 28.39
	150 mg/kg	7.34 ± 0.53	0.85 ± 0.07	145.85 ± 27.68
	300 mg/kg	6.95 ± 0.54	0.83 ± 0.08	159.61 ± 33.62

Values are expressed as mean ± S.E.M. (N = 5).

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

Control = received 5% ethanol in 30% acacia.

Table 7. Effect of GS extract on indomethacin-induced gastric lesions in rats.

Group	Ulcer index	Inhibition (%)
Control	2.50 ± 0.17	-
Misoprostol		
100 μg/kg	0.50 ± 0.14*	80
GS extract		
75 mg/kg	1.80 ± 0.20*	28
150 mg/kg	1.71 ± 0.18*	31
300 mg/kg	1.17 ± 0.31*	53

Drugs were orally administered 1 h before induced gastric lesion.

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

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

Control = received 5% ethanol in 30% acacia