RESULTS

1. ANTI-INFLAMMATORY ACTIVITY

1.1 Effect of CP extract and phenylbutazone on EPP-induced ear edema in rats

The inhibitory effect produced by the topical administration of CP extract on EPP-induced rat ear edema was assessed at 15, 30, 60 and 120 min after EPP application as shown in Table 1. The inflammogen EPP, in a dose of 1 mg/ear, evoked a clear edematous response when topically applied on the rat ear. The average edema thickness in the vehicle (EtOH or acetone) treated animal groups amounted to 194 $\pm$ 5.2 and 210 $\pm$ 4.5 μm, respectively at 1 h after application. CP extract in the dose range of 1 - 4 mg/ear produced significant and dose-dependent inhibition of the 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 CP extract was obtained with the highest dose used in this test i.e. 4 mg/ear. This dose showed significant inhibitory effect on edema formation of 63.82%, 61.70%, 60.82% and 55.84% at 15, 30, 60 and 120 min, respectively, after topical application of EPP. Phenylbutazone, the reference NSAIDs, at the doses of 0.25, 0.5 and 1 mg/ear, also exhibited significant inhibitory activity on the edema formation at all determination times. At the dose 1 mg/ear, phenylbutazone produced marked anti-edema activity of 58.82%, 56.43%, 56.19% and 55.29% at 15, 30, 60 and 120 min, respectively.

The log dose-response plots showed good linear relationships for both CP extract and phenylbutazone with a linear correlation coefficient of 0.9991 and 0.9899, respectively (Figure 7). Furthermore, the dose-response lines of the two test substances were closely parallel. The calculated ED_{50} (effective inhibitory dose for 50% inhibition) values of CP extract and phenylbutazone, determined at 1 h after EPP challenge, were 2.34 and 0.82 mg/ear, respectively. The ED_{50} values obtained, show that the reference compound phenylbutazone was about 3 times more potent than CP extract.

Table 1. Inhibitory effect of CP 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 Acetone	-	102±3.6	-	202±4.7	-	210±4.5	-	170±5.4	-
Control EtOH	-	94±4.3	-	188±5.3	-	194±5.2	-	154±6.0	-
Phenylbutazone	0.25	86±4.3*	18.60	162±6.3**	24.69	170±5.4**	23.53	142±4.7**	19.72
	0.5	60±4.2**	41.18	128±4.4**	36.63	136±5.0**	35.24	112±4.4**	34.12
	1	42±3.6***	58.82	88±5.3***	56.43	92±5.3***	56.19	76±4.0***	55.29
CP extract	1	60±3.0**	36.17	124+4.0**	34.04	132±5.3**	31.96	110±4.5**	28.57
	2	46±3.1***	51.06	94±4.3***	50.00	102±4.7***	47.42	84±5.0***	45.45
	4	34±3.1***	63.82	72±3.3***	61.70	76±4.0***	60.82	68±4.4***	55.84

Test drugs were applied topically to both inner and outer surfaces of the ear. Control = received vehicle (Acetone or EtOH) only.

Values are expressed as mean ± S.E.M. (N = 10). Significantly different from control: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

ED = Edema thickness; EDI = Edema inhibition

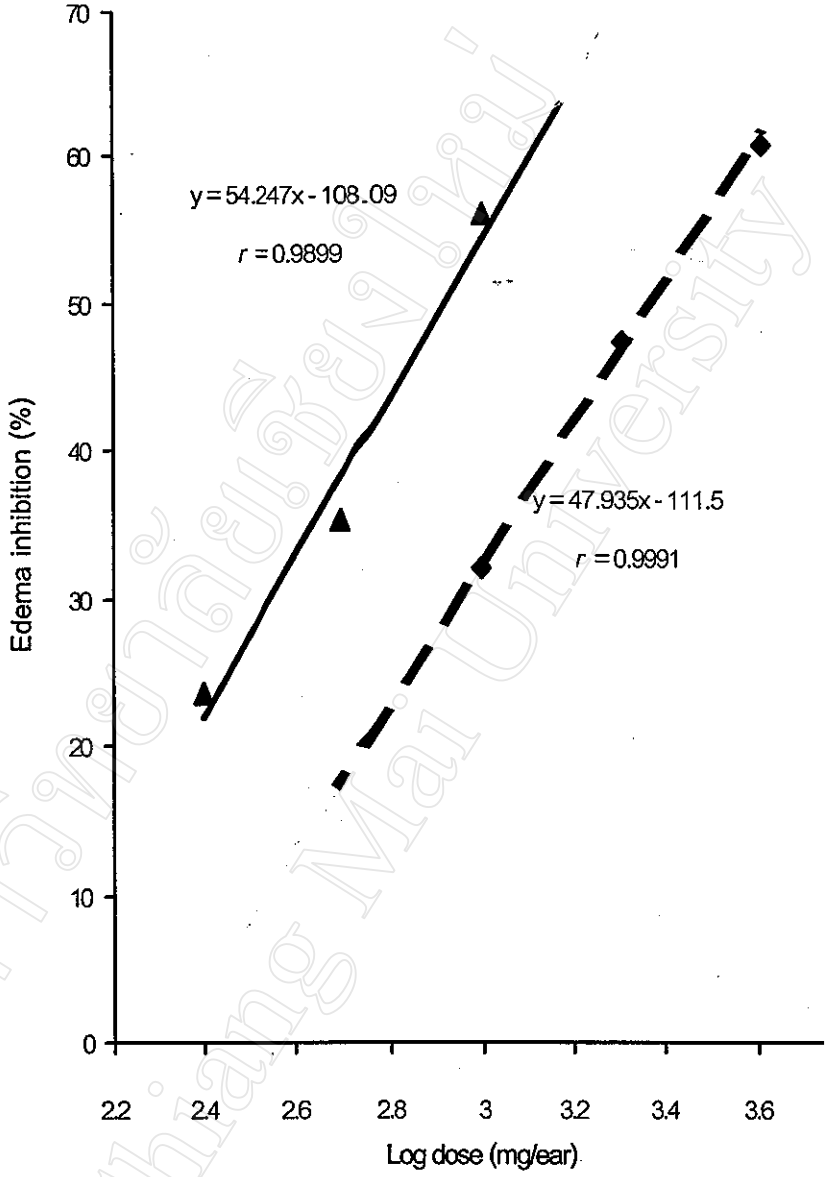

Figure 7. Log dose-response regression line of CP extract (♦) and phenylbutazone (▲) on EPP-induced ear edema.

CP extract ED_{50} = 2.34 mg/ear

Phenylbutazone ED_{50} = 0.82 mg/ear

1.2 Effect of CP 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 CP extract and aspirin after carrageenin injection is shown in Table 2 and Figure 8.

As shown in Table 2, the subplantar injection of carrageenin to control animals produced a local edema that increased progressively to reach a maximal intensity 5 h after the injection of the phlogistic agent. Both CP extract and aspirin were found to exhibit a dose-related inhibition of edema formation. CP extract at doses of 300, 600 and 1200 mg/kg possessed significant inhibitory effects on carrageenin-induced paw edema at all assessment times. Aspirin, a COX inhibitor, at the doses of 75, 150 and 300 mg/kg also exhibited significant edema inhibitory activity. However, the anti-inflammatory effect of CP extract on the paw edema formation at the dose of 300 mg/kg was less effective than that of aspirin at the same dose. The inhibitory effect of CP extract, at a dose of 1200 mg/kg on the paw edema formation, was seen at all determination times similarly to that aspirin at the dose of 300 mg/kg. The percent edema inhibition produced by the dose of 1200 mg/kg of CP extract on carrageenin-induced edema formation of the rat paw were 53.25, 47.79 and 46.11 whereas those produced by the dose of 300 mg/kg of aspirin were 60.50, 54.59 and 51.16 at the 1^{st}, 3^{rd} and 5^{th} h, respectively, after carrageenin injection. Both CP extract and aspirin still possessed the inhibitory effect on the edema formation at 5 h after drug treatment, although this effect was slightly less than that at the 3^{rd} h.

Figure 8 illustrates the dose-response regression lines of CP extract and aspirin, assessed at the third hour after carrageenin challenge. The ED_{30} values of CP extract and aspirin determined from their dose-response curves were found to be 420.41 and 108.54 mg/kg, respectively. The correlation coefficient values of the dose-response relationship of CP extract and aspirin were 0.9936 and 0.9992, respectively. These highly positive correlation coefficient values suggest the dose-dependent inhibitory effect of both compounds on this rat paw edema model. Moreover, it was interesting to note that, the dose-response lines of the two test substances were statistically parallel.

Table 2. Inhibitory effect of CP 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	-	0.38±0.04	-	0.82±0.04	-	0.84±0.03	-
Aspirin	75	0.34±0.03	9.17	0.65±0.05*	20.56	0.70±0.05*	16.74
	150	0.26±0.02*	31.66	0.50±0.04***	38.67	0.53±0.05***	36.94
	300	0.15±0.03**	60.50	0.37±0.04***	54.59	0.41±0.04***	51.16
CP extract	300	0.25±0.04*	33.14	0.63±0.06*	23.21	0.65±0.06*	22.06
	600	0.20±0.02**	46.15	0.51±0.05***	37.92	0.54±0.06***	35.08
	1200	0.18±0.03**	53.25	0.43±0.04***	47.79	0.45±0.03***	46.11

Test drugs were orally administered 1 h before carrageenin injection. Control = received 5% Tween 80 only.

Values are expressed as mean ± S.E.M. ($N = 6$). Significantly different from control: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

EV = Edema volume; EI = Edema inhibition

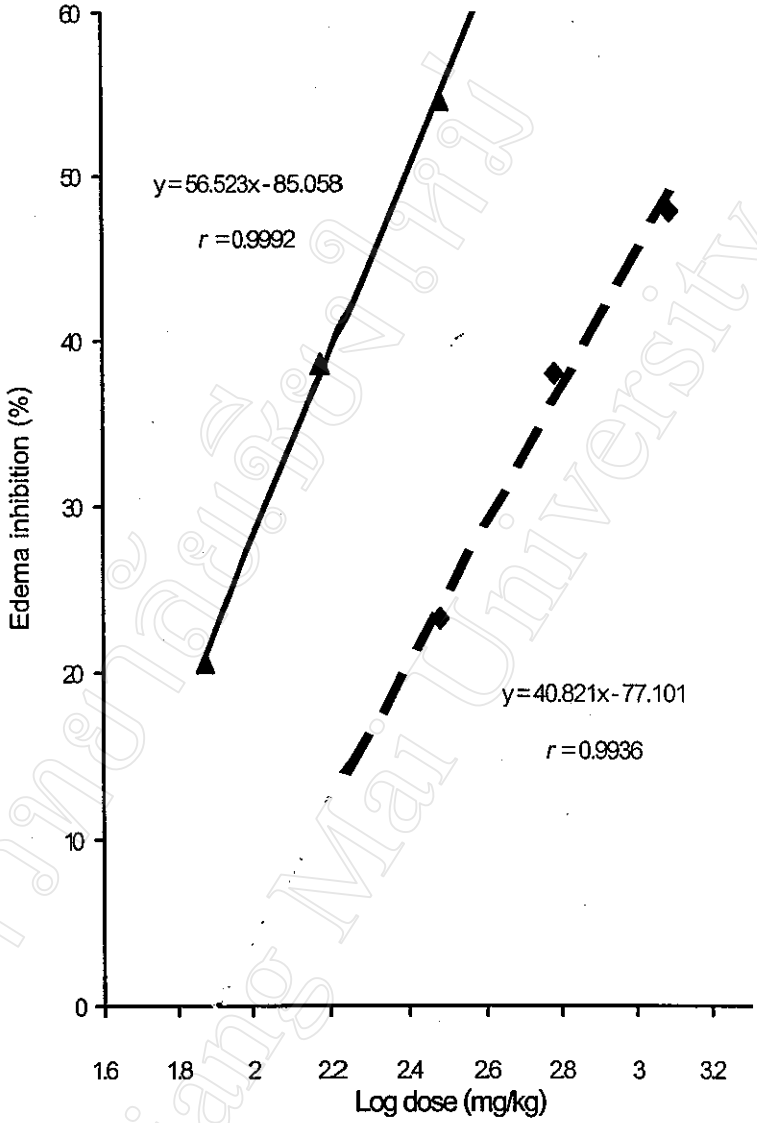

Figure 8. Log dose-response regression line of CP extract (♦) and aspirin (▲) on carrageenin-induced paw edema.

CP extract ED_{30} = 420.41 mg/kg

Aspirin ED_{30} = 108.54 mg/kg

1.3 Effect of CP extract, aspirin and phenidone on AA-induced hind paw edema in rats

Results obtained from the hind paw edema induced by AA are demonstrated in Table 3. AA evoked a severe edematous response when it was injected into the plantar side of the right hind paw. The average edema volume in the vehicle treated group amounted to 0.58 ± 0.03 ml, at 1 h after AA injection. At doses of 300, 600 and 1200 mg/kg, CP extract did not possess significant inhibitory effect on AA-induced hind paw edema. Similarly, aspirin at the dose of 300 mg/kg did not show any inhibitory effect on AA-induced edema. By contrast, phenidone, a dual inhibitor of AA metabolism, exhibited marked inhibitory activity on the edema formation of 47.68% when assessment was done 1 h after injection. The results showed that CP extract had no inhibitory effect on the edema formation induced by AA.

1.4 Effect of CP extract, aspirin and prednisolone on the cotton pellet-induced granuloma formation

1.4.1 Effects on granuloma formation and transudation

The inhibitory effect of CP 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 inhibitory action of CP extract and the reference drugs against granuloma formation induced by cotton pellet implantation is shown in Table 4.1. It was found that CP extract and aspirin, at the dose of 1200 and 300 mg/kg, respectively, elicited only nonsignificant inhibition on the granuloma formation. In contrast to both CP extract and aspirin, prednisolone, the steriodal anti-inflammatory drug, at the dose of 5 mg/kg exhibited significantly inhibitory effect on the granuloma formation. The granuloma inhibitory effect of prednisolone was found to be 40.85%.

The transudative weight in control group was found to be 340.18 ± 12.18 mg. All test substances, CP extract, aspirin and prednisolone, significantly reduced the weight of the transudate to 307.53 ± 7.53, 299.23 ± 12.55 and 230.43 ± 7.55 mg, respectively (Table 4.1).

Table 3. Inhibitory effect of CP extract, aspirin and phenidone on arachidonic acid (AA)-induced paw edema in rats.

Group	Dose (mg/kg)	EV(ml)	EI(%)
Control	-	0.58±0.03	-
Aspirin	300	0.52±0.03	9.85
Phenidone	40	0.30±0.02***	47.68
CP extract	300	0.52±0.04	9.75
	600	0.48±0.03	15.83
	1200	0.49±0.04	14.48

Test drugs were orally administered 2 h before AA injection. The 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: *** $p < 0.001$.

Control = received 5% Tween 80 only.

EV = Edema Volume; EI = Edema inhibition

Table 4. Effects of CP extract, aspirin 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	-	405.93±13.95	65.75±2.31	340.18±12.18	2.27±0.11	-
Aspirin	300	358.14±12.04*	58.92±3.87	299.23±12.55*	1.95±0.19	14.15
Prednisolone	5	277.46± 8.65***	47.03±1.47***	230.43± 7.55***	1.35±0.07***	40.85
CP extract	1200	370.15± 6.4*	62.62±1.82	307.53±7.53*	2.14±0.09	5.83

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

Significantly different from control: * $p < 0.05$; *** $p < 0.001$

GI = Granuloma inhibition. Control = received 5% Tween 80 only.

1.4.2 Effects on body weight gain and thymus weight

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 37.50 $\pm$ 2.92 g. Both CP extract (1200 mg/kg) and aspirin (300 mg/mg), did not affect the body weight gain of animals. The gain of the weight in CP extract and aspirin treated groups were 36.83 $\pm$ 4.72 and 33.17 $\pm$ 2.81 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 16.00 $\pm$ 4.65 g.

Dry thymus weight of rats in control group was 56.58 $\pm$ 2.59 mg/100 g body weight. Both CP extract and aspirin did not showed any suppressive effect on the thymus weight (57.72 $\pm$ 2.87 and 51.69 $\pm$ 2.02 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 22.03 $\pm$ 1.83 mg/100 g body weight.

1.4.3 Effects on alkaline phosphatase activity

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 (53.02 x 10^{-4} U of enz./mg of serum protein) when compared with that of normal or non-implanted rats (29.01 x 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 CP extract at the dose of 1200 mg/kg (33.51 x 10^{-4} U of enz./mg of serum protein) as well as by both aspirin at the dose of 300 mg/kg (37.53 x 10^{-4} U of enz./mg of serum protein) and prednisolone at the dose of 5 mg/kg (30.50 x 10^{-4} U of enz./mg of serum protein).

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	-	197.33±6.23	234.83±6.56	37.50±2.92	56.58±2.59
Aspirin	300	192.33±4.85	225.50±4.70	33.17±2.81	51.69±2.02
Prednisolone	5	193.67±5.67	209.67±7.03*	16.00±4.65**	22.03±1.83***
CP extract	1200	190.33±3.67	227.17±7.62	36.83±4.72	57.72±2.87

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

Significantly different from control: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Control = received 5% Tween 80 only.

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	-	153±20.25	5.3±0.11	29.01±4.18
Control	-	274±20.85	5.3±0.18	53.02±4.96 [a]
Aspirin	300	186±20.46	5.0±0.16	37.53±4.43 [b]
Prednisolone	5	165±13.72	5.4±0.09	30.50±2.55 [c]
CP extract	1200	180±14.93	5.4±0.10	33.51±3.31 [c]

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

a = significant from normal: $p < 0.01$.

b = significant from control: $p < 0.05$.

c = significant from control: $p < 0.01$.

Normal = non-implanted group; Control = implanted group, received 5% Tween 80 only.

2. ULCEROGENIC ACTIVITY

Three groups of six rats each were medicated once daily with test drugs for 7 consecutive days. On the 8th day, the animals were sacrificed and the stomachs were examined with a dissecting microscope for the presence of ulcer in the gastric mucosa.

The averages of gastric lesions caused by test substances are shown in Table 5. The results obtained were compared with vehicle-treated rats. CP extract, at the dose of 1200 mg/kg/day, did not elicited gastric ulceration. On the contrary, aspirin at the dose of 300 mg/kg/day and prednisolone at the dose of 5 mg/kg/day could cause significant gastric ulcers. However, the gastric lesion produced by prednisolone was significantly lesser than that of aspirin.

3. ANALGESIC ACTIVITY

3.1 Effect of CP extract and aspirin on acetic acid-induced writhing response in mice

Writhes were induced in mice by an intraperitoneal injection of 0.75% acetic acid aqueous solution. CP extract and aspirin were administered orally 1 h before the acetic acid injection.

The results of the acetic acid-evoked writhing response in mice are given in Table 6. In the control group, the intraperitoneal injection of acetic acid produced 64.83 $\pm$ 2.4 writhes for 15 min after injection. At doses of 300, 600 and 1200 mg/kg, CP extract did not possess significant inhibitory activity on writhing response. In contrast, aspirin at a dose of 300 mg/kg exerted significant inhibition on the number of writhes with the percentage of inhibition of 80.98.

Table 5. Effect of CP extract, aspirin and prednisolone on gastric mucosa

Group	Dose (mg/kg)	Gastric lesions (mm)
Control	-	0
Aspirin	300	10.50±1.59***
Prednisolone	5	4.17±1.87*
CP extract	1200	0

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

Significantly different from control: * $p < 0.05$; *** $p < 0.001$

Control = received 5% Tween 80 only.

Table 6. Inhibitory activity of CP extract and aspirin on writhing response in mice

Group	Dose (mg/kg)	No. of writhes	Decrease of Writhing Response (%)
Control	-	64.83±2.4	-
Aspirin	300	12.33±1.4***	80.98
CP extract	300	57.67±1.6	10.05
	600	57.33±2.3	11.57
	1200	53.83±4.1	16.97

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

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

Significantly different from control: *** $p < 0.001$.

Control = received 5% Tween 80 only.

4. ANTIPYRETIC ACTIVITY

4.1 Yeast-induced hyperthermia in rats

As shown in Table 7 and Figure 9, significant reduction of the rectal temperature was observed at all assessment times in the group of rats, which received aspirin. The significant decrease of rectal temperature was also observed in the rats treated with CP extract. It was found that the CP extract possessed the same antipyretic potency as that of aspirin. At the dose of 300 mg/kg, aspirin could reduce the rectal temperature of the rats to 38.45 $\pm$ 0.1, 38.06 $\pm$ 0.1, 37.70 $\pm$ 0.2 and 37.50 $\pm$ 0.2 when measurement was made at 30, 60, 90 and 120 min, respectively, after drug administration. The CP extract, with an equal dose to aspirin (300 mg/kg), reduced the hyperthermia to 38.28 $\pm$ 0.2, 37.94 $\pm$ 0.3, 37.73 $\pm$ 0.3 and 37.60 $\pm$ 0.3 at 30, 60, 90 and 120 minutes, respectively after an extract administration.

5. HIPPOCRATIC ACTIVITY

This part of the study was used to investigate the changes in general behavior, which could be observed in the conscious animals after receiving large doses of test drugs.

Two groups of five rats each were orally medicated with CP extract and vehicle. Vehicle (5% Tween 80)-treated (5 ml/kg) group served as control. In comparison with the control group, CP extract, at the dose of 5000 mg/kg, did not cause death of animals. It did not produce any signs and symptoms of toxicity or any changes of general behavior of the rats. Moreover, it was found that the normal body weight gain of the animal was not affected by CP extract during the observation period of 8 days. Therefore, other doses of CP extract were not tested, as according to OECD guidelines for testing of chemicals, if a dose of 5000 mg/kg did not cause death of animals, the test substance is considered as non-toxic. All animals were killed and necropsied after 8 days of observation. No change in either color or size of the internal organs (heart, liver, lung, spleen, gastrointestinal tract, genitalia as well as brain) was seen in both groups of animals.

Table 7. Effect of CP extract and aspirin on yeast- induced hyperthermia in rats

Group	Dose (mg/kg)	Body weight (g)	Rectal temperature (°C)					
			Before Yeast injection	18 h after yeast injection				
				Time after medication (min)				
				0	30	60	90	120
Control	-	211.00±6.2	37.56±0.1	39.05±0.1	38.93±0.2	38.93±0.1	38.89±0.1	38.85±0.1
Aspirin	300	214.00±4.4	37.26±0.1	39.31±0.1	38.45±0.1*	38.06±0.1**	37.70±0.2***	37.50±0.2***
CP extract	300	216.50±4.8	37.16±0.2	39.14±0.1	38.28±0.2**	37.94±0.3**	37.73±0.3***	37.60±0.3***

Test drugs were orally administered 18 h after yeast injection.

Values are expressed as mean ± S.E.M. ($N = 8$).

Significantly different from the rectal temperature after yeast injection 18 h: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Control = received 5% Tween 80 only.

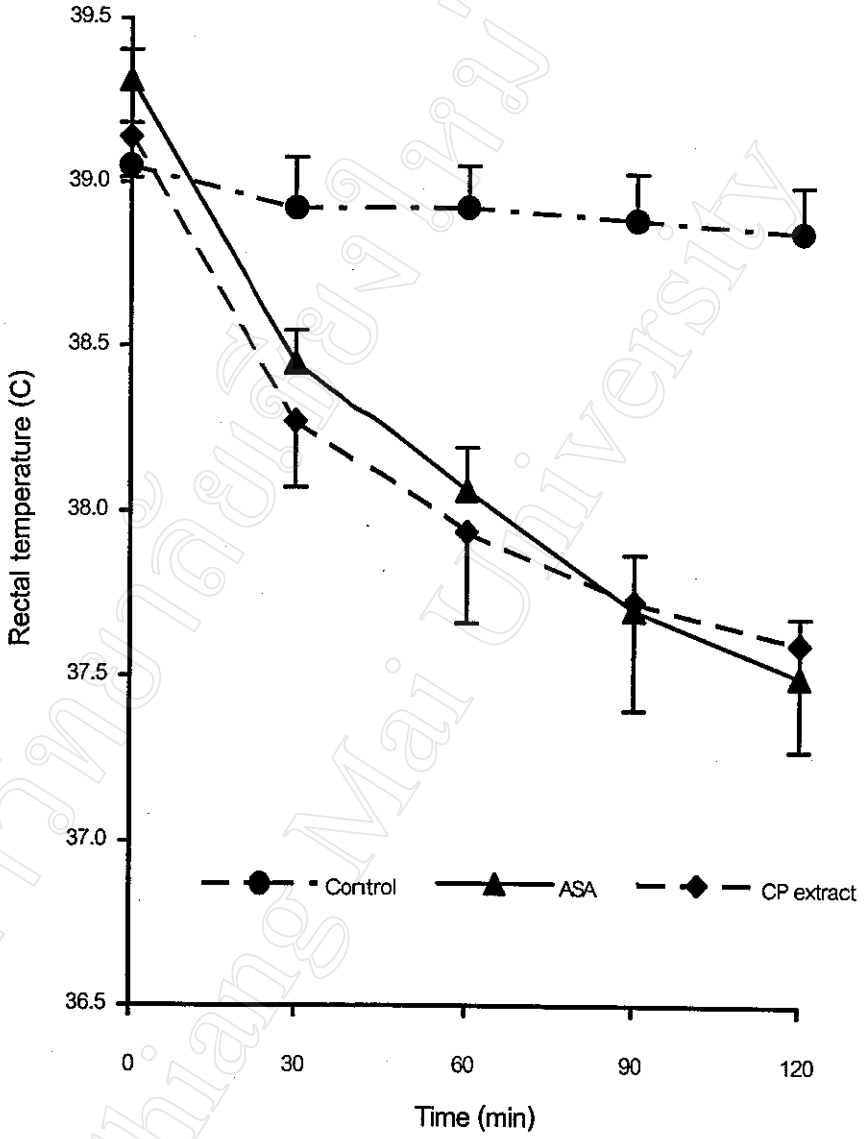

Figure 9. Effect of CP extract and aspirin on yeast-induced hyperthermia in rats. Test drugs (300 mg/kg) were given orally 18 h after yeast injection.