RESULTS

1. Effect of Ochna extracts, aspirin and morphine on acetic acid-induced writhing response in mice

The abdominal constriction response is thought to involve, in part, local peritoneal receptors. Writhes were induced in mice by an intraperitoneally injection of 0.75% acetic acid aqueous solution. Ochna extracts, morphine and aspirin were administered intraperitoneally 30 min before the acetic acid injection.

As shown in Table 1 and Figure 5 morphine at a dose of 10 mg/kg completely inhibited the writhing response. Administration of aspirin at doses of 37.5, 75 and 150 mg/kg also revealed significant inhibition on the number of writhes induced in mice by acetic acid with the percentage of inhibition of 48, 76, 95, respectively. The inhibitory effect of aspirin was found to be dose-related ($r = 0.995$). The effective inhibitory dose at 50% (ED_{50}) of aspirin determined from its dose-response curve (Figure. 6) was found to be 38 mg/kg.

The OI-LF was found to possess profound inhibitory activity on writhing response. At doses of 37.5, 75, and 150 mg/kg, OI-LF showed reduction of writhes with the percentage of 20, 45, 95, respectively. The inhibitory effect on the writhing response of OI-LF was dose-related with the r value of 0.980. The ED_{50} value of OI-LF was found to be 71 mg/kg.

The OI-TW, at the same doses as aspirin caused the reduction of writhes with the percentage of 24, 62, and 96, respectively. The ED_{50} value of OI-TW was found to be 61 mg/kg.

The OI-ST showed the decrease of writhing response with the percentage of 42, 64, 93 when doses of 37.5, 75 and 150 mg/kg, respectively were used. The r value of the dose-response relationship of OI-ST was found to be 0.996. From the log dose-response regression line, the ED_{50} of the OI-ST was found to be 48 mg/kg.

The OI-BK was found to possess the pronounced inhibitory effect on the writhing response. At the same doses as other extracts, OI-BK showed the reduction of writhes with the percentage of 48, 76, 97, respectively. The inhibitory effect on the writhing response of OI-BK was dose-related with the r value of 0.997 and the ED_{50} value of 38 mg/kg.

Table 1. Effect of Ochna extracts, morphine and aspirin on acetic acid-induced writhing response in mice

Group	Dose (mg/kg)	No. of writhes	Inhibition (%)
Control	-	45.2 ± 1.4	-
Morphine	10	0.0	100
Aspirin	37.5	23.7 ± 1.0*	48
	75	11.0 ± 0.9*	76
	150	2.2 ± 0.3*	95
OI-LF	37.5	36.2 ± 1.4*	20
	75	24.8 ± 2.0*	45
	150	2.2 ± 0.3*	95
OI-TW	37.5	34.3 ± 2.3*	24
	75	17.2 ± 2.0*	62
	150	1.8 ± 0.3*	96
OI-ST	37.5	26.0 ± 1.6*	42
	75	16.4 ± 2.0*	64
	150	3.1 ± 1.3*	93
OI-BK	37.5	23.3 ± 1.6*	48
	75	11.0 ± 1.0*	76
	150	1.2 ± 0.5*	97

Test drugs were administered intraperitoneally 30 min before acetic acid injection. Values were expressed as mean ± S.E.M (n=6).

* Significantly different from control group ($p < 0.05$)

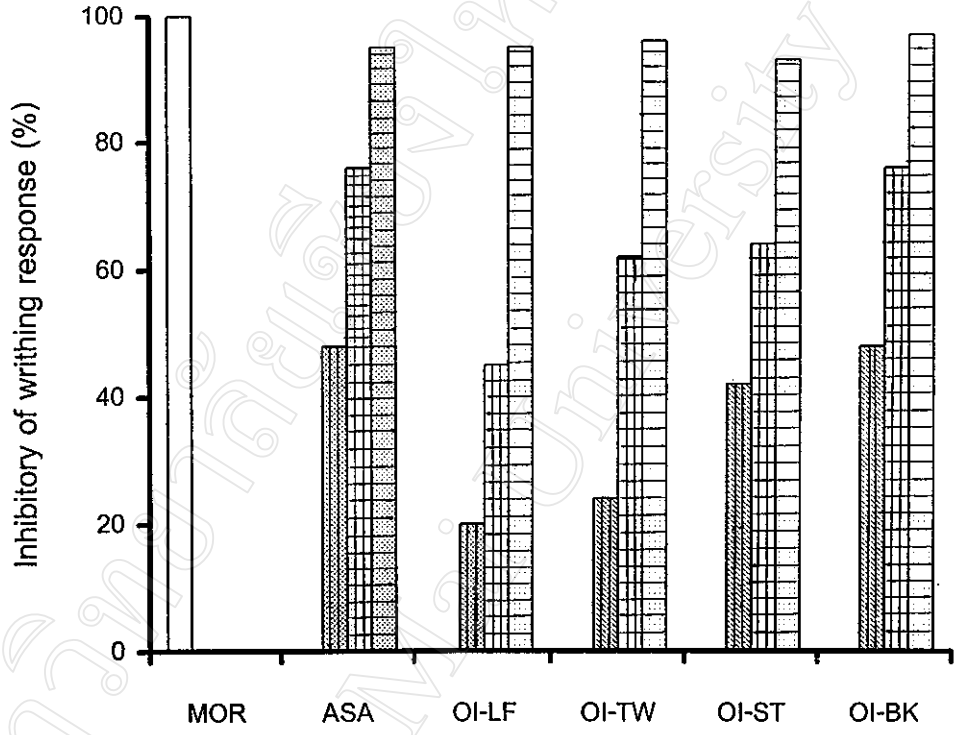

Figure 5. Histogram of the inhibitory effect of Ochna extracts, morphine and aspirin on acetic acid-induced writhing response in mice. Test drugs were given intraperitoneally 30 min before acetic acid injection.

Symbol; 10 mg/kg (☐), 37.5 mg/kg (▥), 75 mg/kg (▦), 150 mg/kg (▤)

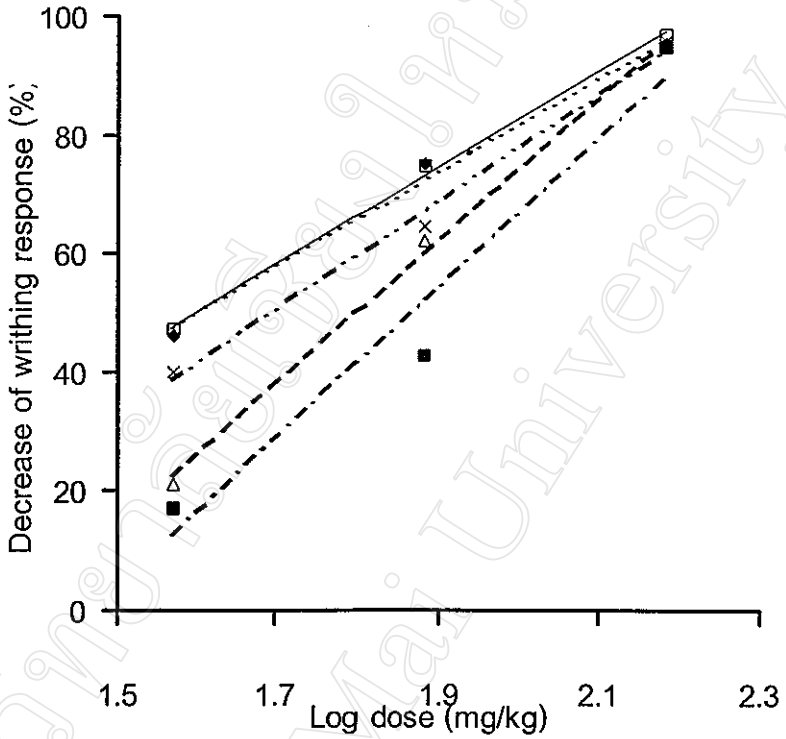

Figure 6. Log dose-response regression line of Ochna extracts and aspirin on the acetic acid-induced writhing response in mice.

Symbol; Aspirin (◆), OI-LF(■), OI-TW(△), OI-ST(✳), OI-BK(□).

Aspirin	Y = 77.12x – 71.73.	r = 0.995	ED_{50} = 38mg/kg
OI-LF	Y = 122.72x - 176.96.	r = 0.980	ED_{50} = 71 mg/kg
OI-TW	Y = 118.06x – 160.89.	r = 0.999	ED_{50} = 61 mg/kg
OI-ST	Y = 83.54x – 90.44.	r = 0.996	ED_{50} = 48 mg/kg
OI-BK	Y = 80.83x – 77.19.	r = 0.997	ED_{50} = 38 mg/kg

2. Effect of Ochna extracts, morphine and aspirin on the rat tail-flick reflex

Pain on the rat's tail was induced by applying the heat from 55 °C water. The rat flicked its tail when it felt pain. A 30 s cut-off time was set in order to avoid any damage to the tails. Animals with a control reaction time more than 2 s were rejected.

The inhibitory effects of Ochna extracts, aspirin and morphine on the tail-flick reflex in rats are shown in Table 2. At a dose of 10 mg/kg, morphine, a centrally acting analgesic drug completely inhibited the tail-flick response at time 30, 60, 90, 120 and 150 min after intraperitoneal injection. Aspirin and OI-LF at a dose of 150 mg/kg possessed no analgesic effect in this test model. Anyhow, the OI-TW at the dose of 150 mg/kg exhibited slightly analgesic effect at time 90 and 120 min on the tail-flick reflex. The OI-ST at the same dose also showed mild analgesic effect at time 120 min. Similarly OI-BK, an extract from bark, showed slight analgesic effect in this test model. It inhibited the tail flick response significantly at 60, 90 and 120 min (Table 2).

Table 2. Effect of Ochna extracts, morphine and aspirin on tail-flick response in mice

Group	Dose (mg/kg)	Reaction time after treatment (s)					
		0	+30	+60	+90	+120	+150 min
Control	-	1.5 ±0.1	1.4 ±0.1	1.8 ±0.1	1.6 ± 0.1	1.6 ± 0.1	1.4 ± 0.2
Morphine	10	1.1 ± 0.1	>30*	>30*	>30*	>30*	16.0 ± 1.1*
Aspirin	150	1.1 ± 0.1	1.4 ± 0.2	1.7 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	1.5 ± 0.1
OI-LF	150	1.2 ± 0.1	1.5 ± 0.2	1.5 ± 0.1	2.0 ± 0.2	1.6 ± 0.1	1.7 ± 0.2
OI-TW	150	1.1 ± 0.1	1.5 ± 0.1	1.7 ± 0.2	3.1 ± 0.2*	2.3 ± 0.1*	2.0 ± 0.3
OI-ST	150	1.1 ± 0.1	1.6 ± 0.2	1.3 ± 0.1	1.6 ± 0.2	2.1 ± 0.2*	1.6 ± 0.2
OI-BK	150	1.1 ± 0.1	1.8 ± 0.2	2.9 ± 0.3*	2.1 ± 0.2*	2.1 ± 0.2*	2.0 ± 0.2

Test drugs were administered intraperitoneally, tail flick response was measured at 30, 60, 90, 120 and 150 min. Values were expressed as mean ± S.E.M (n=6). * Significantly different from control group ($p < 0.05$).

3. Effect of Ochna extracts morphine and aspirin on the formalin test in mice

An injection of formalin at the right dorsal hindpaw induces pain by evoking sensory C fiber in the early phase and enhances inflammatory process in the late phase. In this test model the intensive licking time was used as a criterion for indicating pain. The results obtained at the early phase as shown in Table 3 and Figure 7 showed that morphine at a dose of 10 mg/kg completely inhibited licking response. Aspirin at a dose of 150 mg/kg could inhibit the licking response with the percentage of 27. The Ochna extracts at a dose of 150 mg/kg, similarly showing analgesic activity with higher intensity than aspirin, possessed inhibitory effect on licking response with the percentage of 41, 55, 61, and 75, respectively.

Results obtained at the late phase of formalin test (Table 4 and Figure 7) revealed the complete inhibitory effect of morphine as shown by no licking at all after injection of morphine at a dose of 10 mg/kg. Aspirin at a dose of 150 mg/kg elicited analgesic effect on this test model with the percentage of inhibition on licking of 53. Similar to aspirin, the OI-LF, at a dose of 150 mg/kg exhibited moderate analgesic effect with the percentage of inhibition on licking of 50. At a dose of 150 mg/kg, the OI-TW, OI-ST and OI-BK exhibited an intensive analgesic activity with percentage of inhibition 74, 77 and 81, respectively.

Table 3. Effect of Ochna extracts, morphine and aspirin on the early phase of formalin test in mice

Group	Dose (mg/kg)	Licking time[a] (s)	Inhibition (%)
Control	-	57.5 ± 0.8	-
Morphine	10	0.0*	100
Aspirin	150	41.8 ± 2.4*	27
OI-LF	150	34.2 ± 1.1*	41
OI-TW	150	25.8 ± 1.7*	55
OI-ST	150	22.3 ± 1.6*	61
OI-BK	150	14.2 ± 1.4*	75

Test drugs were administered intraperitoneally 30 min before 1% formalin injection. Values were expressed as mean ± S.E.M (n=6). * Significantly different from control group ($p < 0.05$). [a] Seconds between 0-5 min after formalin injection.

Table 4. Effect of Ochna extracts, morphine and aspirin on the late phase of formalin test in mice

Group	Dose (mg/kg)	Licking time[a] (s)	Inhibition (%)
Control	-	67.8 ± 3.2	-
Morphine	10	0.0*	100
Aspirin	150	31.8 ± 2.3*	53
OI-LF	150	33.8 ± 2.6*	50
OI-TW	150	17.7 ± 2.5*	74
OI-ST	150	15.7 ± 0.9*	77
OI-BK	150	12.8 ± 1.2*	81

Test drugs were administered intraperitoneally 30 min before 1% formalin injection. Value were expressed as mean ± S.E.M (n=6). * Significantly different from control group ($p < 0.05$). [a] Seconds between 20-30 min after formalin injection.

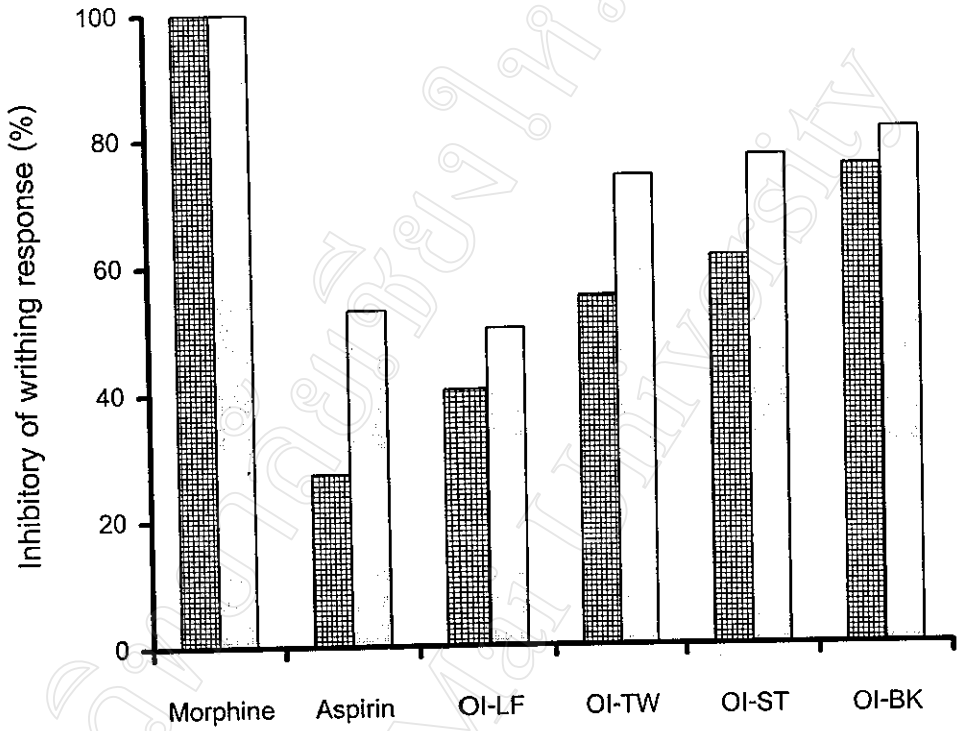

Figure 7. Histogram of the inhibitory effect of Ochna extracts, morphine and aspirin on the early and late phase of formalin test in mice. Test drugs were given intraperitoneally.

Symbol; early phase (▦), late phase (□).

4 Effect of Ochna extracts, phenylbutazone and phenidone, on ear edema in rats

4.1 Ethyl phenylpropiolate (EPP)-induced edema formation

Results obtained from the EPP-induced rat ear-edema are summarized in Table 5. Phenylbutazone, a potent nonsteroidal anti-inflammatory drug, at the dose of 1 mg/ear inhibited the edema formation significantly. The pronounced inhibitory effect (51 and 50%) of phenylbutazone was obviously seen by the first measurement at 30 and 60 min after application of EPP. The percentages of edema inhibition were slightly lower to 47 when measurement was made at 120 min after EPP application.

The OI-LF at a dose of 4 mg/ear significantly inhibited the ear edema formation with the percentage of inhibition of 46, 57, 52 and 54 at 15, 30, 60 and 120 min. At a dose of 2 mg/ear, the percentage of edema inhibition was found to be 38, 43, 39, and 40, respectively after the edema induction. While at a dose of 1 mg/ear, the OI-LF exerted slightly significant inhibition of the ear edema formation only at time 30 and 60 min with percentage of edema inhibition were 22 and 21.

At a dose of 4 mg/ear, the OI-TW significantly inhibited ear edema formation with percentage of inhibition of 55, 62, 60 and 56, respectively at 15, 30, 60 and 120 min. With the lower dose of 2 mg/ear, the OI-TW also showed significant inhibition of the ear edema formation with percentage of edema inhibition of 46, 54, 48, and 49,

respectively, after the edema induction. While at a dose of 1 mg/ear, the percentage of edema inhibition of the OI-TW was 21, 22 and 24 at time 15, 30, and 60 min, respectively.

The OI-ST an extract from stem, at a dose of 2 mg/ear, showed significant inhibition of ear edema with the percentage of 34, 41, 36 and 38, respectively. At a dose of 4 mg/ear, the OI-ST significantly inhibited the ear edema formation at time 15, 30, 60 and 120 min with the percentage of edema inhibition were 41, 54, 52 and 51, respectively. With the lowest dose used in this test model, 1 mg/ear, OI-ST still exhibited significant anti-edema at time 15, 30, and 60 min with percentage of inhibition were 21, 22 and 21.

At the highest dose used in this test model, 4 mg/ear, the OI-BK markedly inhibited the ear edema formation with the percentage of edema inhibition of 53, 60, 59 and 57, respectively. At the dose of 2 mg/ear, the OI-BK showed the edema inhibition with the percentage 41, 49, 50, and 47 at time 15, 30, 60 and 120 min respectively, after edema induction. While at the lowest dose of 1 mg/ear, the OI-BK also significantly inhibited the ear edema formation as shown in table 5.

4.2 Arachidonic acid (AA)-induced edema formation

Table 6 summarizes the results obtained from the rat ear edema induced by AA, which is maximal at 40-60 min. At the dose of 1 mg/ear phenylbutazone, a cyclooxygenase inhibitor could not inhibit the

Table 5. Effect of topical application of Ochna extracts and phenylbutazone on ethylphenylpropiolate-induced ear edema in rats

Treatment	Dose (mg/ear)	Edema thickness (um)				Inhibition (%)			
		15	30	60	120	15	30	60	120
Control	-	80 ± 5	157 ± 3	193 ± 4	157 ± 3	-	-	-	-
Phenylbutazone	1	43 ± 3*	77 ± 6*	97 ±6*	83 ± 8*	46	51	50	47
OI-LF	1	69 ± 10	123 ± 6*	153 ± 6*	137 ± 12	14	22	21	13
	2	50 ± 3*	90 ± 7*	117 ± 6*	94 ± 9*	38	43	39	40
	4	43 ± 8*	67 ± 4*	93 ± 2*	73 ± 4*	46	57	52	54
OI-TW	1	63 ± 10*	123 ± 10*	147 ± 12*	130 ± 16	21	22	24	17
	2	43 ± 10*	73 ± 8*	100 ± 10*	80 ± 10*	46	54	48	49
	4	36 ± 5*	60 ± 7*	77 ± 9*	66 ± 6*	55	62	60	58

Table 5. (Continue)

Treatment	Dose (mg/ear)	Edema thickness (um)				Inhibition (%)			
		15	30	60	120	15	30	60	120
OI-ST	1	63 ± 6*	123 ± 6*	153 ± 7*	133 ± 7	21	22	21	15
	2	53 ± 7*	93 ± 7*	123 ± 6*	102 ± 6*	34	41	36	35
	4	47 ± 4*	73 ± 7*	93 ± 7*	77 ± 16*	41	54	52	51
OI-BK	1	53 ± 7*	97 ± 10*	133 ± 11*	113 ± 11*	34	38	31	28
	2	47 ± 4*	80 ± 7*	97 ± 10*	83 ± 8*	41	49	50	47
	4	38 ± 3*	63 ± 6*	80 ± 5*	67 ± 4*	53	60	59	57

Values were expressed as mean ± S.E.M (n=6). * Significantly different from control ($p < 0.05$)

edema formation after AA application. In contrast, phenidone, an inhibitor of cyclooxygenase and lipoxygenase, at the dose of 2 mg/ear inhibited edema formation significantly with the percentage of inhibition of 48, 49 and 49 at 30, 60 and 120 min after AA application, respectively.

In this study there is the positive correlation between doses (1, 2 and 4 mg/ear, respectively) of Ochna extracts and edema inhibition. At the dose of 1 mg/ear, all Ochna extracts did not show any anti-edema formation of the ear. Anyhow, at the dose of 2mg/ear OI-LF, OI-ST and OI-BK significantly inhibited the ear edema formation at the time 30, 60 and 120 min after AA application, whereas OI-TW showed slightly nonsignificant inhibitory effect. At the dose of 4 mg/ear, all Ochna extracts significantly inhibited mouse ear edema with the pronounced intensity at all assessment times after AA application. At 60 min where the edema of the ear was at peak, OI-LF, OI-TW, OI-ST and OI-BK showed significant inhibition of edema with the percentage of inhibition 51, 59, 59 and 54, respectively.

Table 6. Effect of topical application of Ochna extracts, phenylbutazone and phenidone on arachidonic acid-induced ear edema in rat

Treatment	Dose (mg/ear)	Edema thickness (um)				Inhibition (%)			
		15	30	60	120	15	30	60	120
Control	-	40 ± 5	90 ± 7	123 ± 6	93 ± 7	-	-	-	-
Phenylbutazone	1	37 ± 3	77 ± 4	103 ± 3	83 ± 4	8	14	16	11
Phenidone	2	40 ± 0	47 ± 4*	63 ± 3*	47 ± 4*	0	48	49	49
OI-LF	1	40 ± 5	77 ± 4	103 ± 3	77 ± 6	0	14	16	17
	2	27 ± 4*	57 ± 6*	77 ± 3*	67 ± 8*	33	37	37	28
	4	20 ± 0*	43 ± 12*	60 ± 5*	47 ± 12*	50	52	51	49

Table 6. (Continue)

Treatment	Dose (mg/ear)	Edema thickness (um)				Inhibition (%)			
		15	30	60	120	15	30	60	120
OI-TW	1	37 ± 3	80 ± 4	110 ± 10	87 ± 11	8	11	11	6
	2	33 ± 4	73 ± 11	100 ± 5	83 ± 11	18	19	19	11
	4	20 ± 5*	36 ± 4*	50 ± 4*	40 ± 14*	50	60	59	57
OI-ST	1	40 ± 5	77 ± 3	107 ± 4	87 ± 4	0	14	13	6
	2	30 ± 10	50 ± 11*	70 ± 11*	53 ± 7*	25	44	43	43
	4	27 ± 4*	40 ± 0*	50 ± 4*	47 ± 10*	33	56	59	49
OI-BK	1	33 ± 4	73 ± 6	100 ± 5	77 ± 6	18	19	19	17
	2	31 ± 3	59 ± 3*	77 ± 11*	67 ± 7*	23	34	37	28
	4	20 ± 0*	43 ± 12*	57 ± 12*	40 ± 14*	50	52	54	57

Values were expressed as mean ± S.E.M (n=6). * Significantly different from control ($p < 0.05$)

6. Yeast-induced hyperthermia in rats

20% Brewers yeast suspension in normal saline was used to induce hyperthermia by subcutaneous injection at the back of the rat's neck. Rectal temperature was measured at 18 hours after injection; only animals that showed an increase in the body temperature of not less than 1 °C were intraperitoneally administered of the test agents. The rectal temperature was measured every 30 min for 2 h after test agent administration.

As shown in Table 7 and Figure 8, in the group of rats, which received aspirin at the dose of 150 mg/kg, significant reduction of the rectal temperature of hyperthermic rats was observed. More significant decrease of rectal temperature was observed with the Ochna extracts. It was also found that the extracts possessed the antipyretic activity as aspirin did. At a dose of 150 mg/kg, aspirin could reduce the rectal temperature of the rats to 38.4 $\pm$ 0.1, 38.1 $\pm$ 0.2 and 37.9 $\pm$ 0.3 when measurement was made 60, 90 and 120 min, respectively, after drug administration. The OI-LF, at a dose of 150 mg/kg reduced the hyperthermia to 38.4 $\pm$ 0.3, 38.0 $\pm$ 0.3, 37.8 $\pm$ 0.3 and 37.7 $\pm$ 0.3 at 30, 60, 90 and 120 minutes, respectively, after an extract administration. At the same dose of 150 mg/kg, OI-TW decreased rat's rectal temperature to 38.4 $\pm$ 0.2, 38.0 $\pm$ 0.3, 37.6 $\pm$ 0.4 and 37.4 $\pm$ 0.5 at 30, 60, 90 and 120 minutes, respectively. An extract from stem, OI-ST, with an equal dose to aspirin, reduced the temperature of hyperthermic rats to 38.3 $\pm$

0.3, 37.7 ± 0.3, 37.3 ± 0.2 and 37.1 ± 0.3 at 30, 60, 90 and 120 min, respectively after an extract administration.

As shown in figure 8, OI-BK showed the pronounced effect in reduction of rectal temperature after yeast-induced body temperature. At a dose of 150 mg/kg, the OI-BK reduced the rat's rectal temperature to 38.0 ± 0.3, 37.3 ± 0.4, 37.0 ± 0.5 and 36.3 ± 0.5 at 30, 60, 90 and 120 min, respectively, after an extract administration.

6. Toxicity test and LD_{50} determination.

The LD_{50} was assumed by using 50% death within 72 h following intraperitoneally injection of OI-BK, which showed highly analgesic and antipyretic activities. Male mice weighing 30-40 g were used in this experiment. The OI-BK produced 100% lethality when a dose of 400 mg/kg or more were administered intraperitoneally. At a dose of 350 mg/kg, the OI-BK showed 80% lethality. The 70% lethality was observed at a dose of 300 mg/kg. While at the doses of 250 and 200 mg/kg the percentage of lethality of mice was found to be 40 and 20. The bark extract at a dose of 150 kg/mg did not induce any deaths; therefore by using Litchfield and Wilcoxon method, the LD_{50} was estimated to be about 258 mg/kg. Intense tremors and tonic convulsion before death were observed at the doses of 400 mg/kg or more. Sedation was observed after injection of this extract.

Table 7. Effect of Ochna extracts and aspirin on yeast-induced hyperthermia in rats

Group	Dose (mg/kg)	Rectal temperature (°C)					
		Before yeast injection	18 h after yeast injection	Time after medication (min)			
				30	60	90	120
Control	-	38.2 ± 0.1	39.3 ± 0.2	39.3 ± 0.1	39.3 ± 0.1	39.4 ± 0.2	40.0 ± 0.1
Aspirin	150	38.4 ± 0.1	39.6 ± 0.1	38.9 ± 0.1	38.4 ± 0.1*	38.1 ± 0.2*	37.9 ± 0.3*
OI-LF	150	38.4 ± 0.2	39.4 ± 0.1	38.4 ± 0.3*	38.0 ± 0.3*	37.8 ± 0.3*	37.7 ± 0.3*
OI-TW	150	37.8 ± 0.3	39.2 ± 0.3	38.4 ± 0.2*	38.0 ± 0.3*	37.6 ± 0.4*	37.4 ± 0.5*
OI-ST	150	37.8 ± 0.3	39.3 ± 0.3	38.3 ± 0.3*	37.7 ± 0.3*	37.3 ± 0.2*	37.1 ± 0.3*
OI-BK	150	37.0 ± 0.3	39.0 ± 0.1	38.0 ± 0.3*	37.3 ± 0.4*	37.0 ± 0.5*	36.3 ± 0.5*

Drugs were given (i.p.) 18 h after yeast injection. Values were expressed as mean ± S.E.M (n=6). * Significantly different from the rectal temperature after yeast injection 18 h ($p < 0.05$)

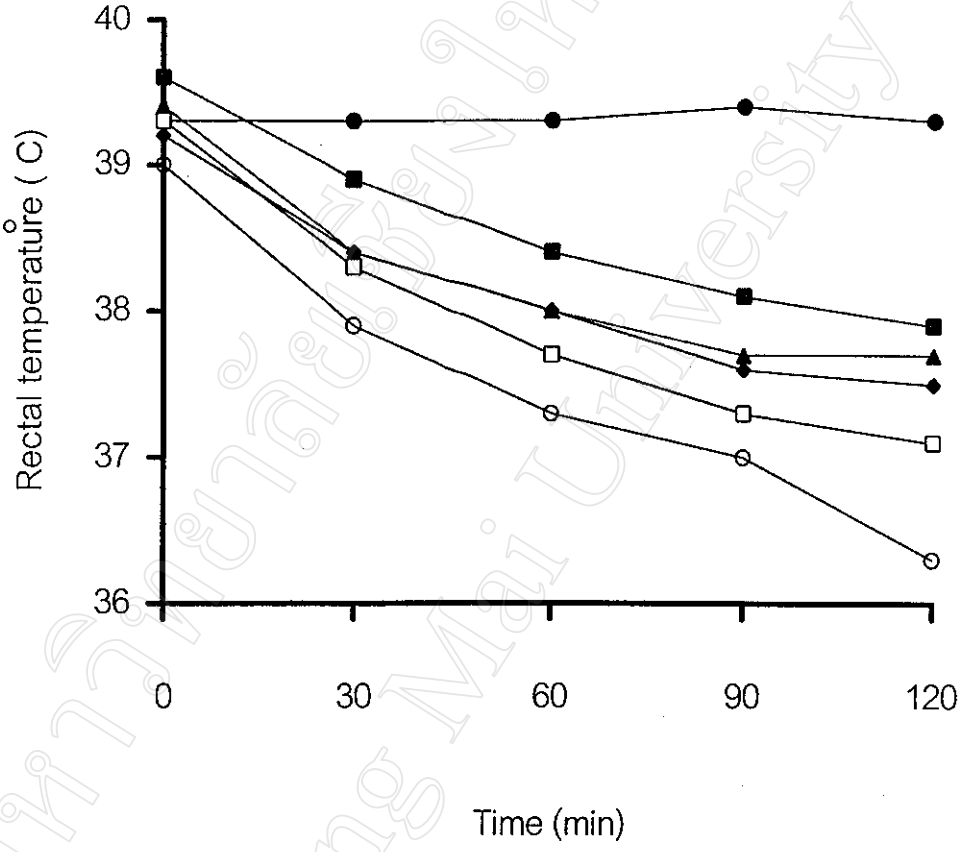

Figure 8. Effect of Ochna extracts and aspirin on yeast-induced hyperthermia in rats. Test drugs (150 mg/kg) were given intraperitoneally 18 h after yeast injection.

Symbol; Control (—○—), Aspirin (—■—), OI-LF (—▲—), OI-TW (—◆—) OI-ST (—□—) and OI-BK (—●—).

Table 8. Percent lethality of mice caused by various doses of the extract from bark of *O. integerrima* (OI-BK)

Dose (mg/kg)	n	no. of death	Lethality (%)
150	10	0	0
200	10	2	20
250	10	4	40
300	10	7	70
350	10	9	90
400	10	10	100

OI-BK was administered intraperitoneally, lethality was observed after 72 h an extract injection

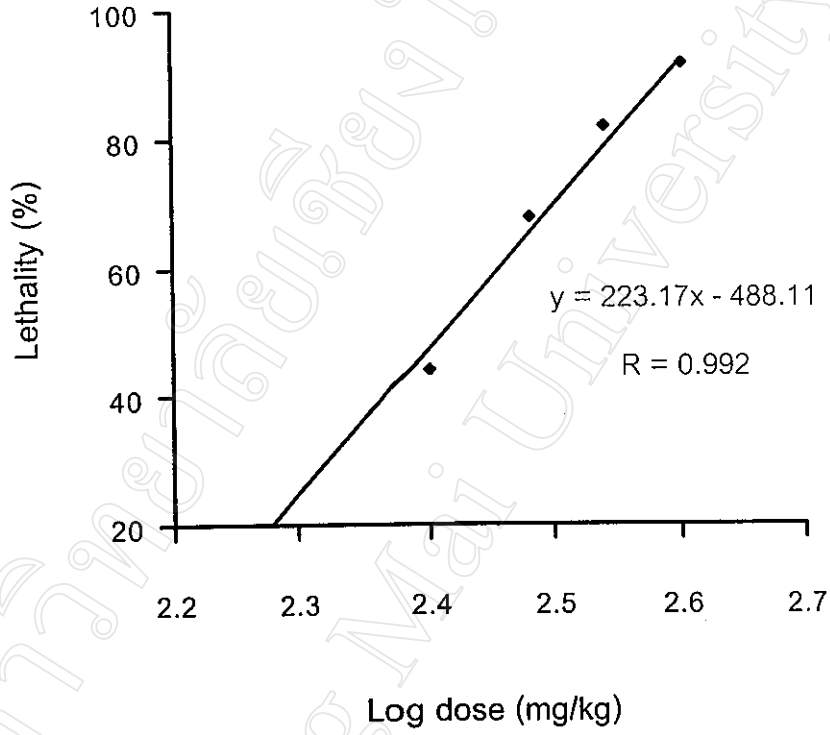

Figure 9. Log dose-response line of the extract from bark of O. integerrima (OI-BK) on lethality of mice within 72 h after intraperitoneally injection

$Y = 223.17x - 488.11$ $r = 0.992$. $LD_{50} = 258$ mg/kg