RESULTS

1. Preliminary screening

Writhing response in mice was used for preliminary screening of analgesic activity of the crude methanol extract of *D. variegata*.

Writhes were induced in mice by intraperitoneal injection of 0.75% acetic acid. Crude methanol extract, morphine and aspirin were given intraperitoneally 30 min before acetic acid injection.

The result obtained, as shown in Table 1, revealed that in the group of mice receiving morphine 10 mg/kg that the writhing response was completely inhibited. It was also found that in the group of mice receiving aspirin at doses of 37.5, 75, and 150 mg/kg, significant inhibitory effect on the writhing response with the percentage of inhibition of 20, 65 and 88, respectively, were observed. Crude methanol extract of *D. variegata* at doses of 75 and 150 mg/kg significantly inhibited writhing response with the percentage of inhibition of 45 and 67, respectively. At the dose of 37.5 mg/kg, crude methanol extract did not show inhibitory activity on the number of writhes. As the methanol extract obtained seemed to separate into oily part, it was therefore partitioned with hexane to give

Table 1. Effect of aspirin, morphine and crude methanol extract from *D. variegata* on acetic acid-induced writhing response in mice

Group	Dose (mg/kg)	No. of writhes	Inhibition (%)
Control	-	60.17 ± 4.27	-
Aspirin	37.5	48.33 ± 2.43*	20
	75.0	20.50 ± 2.51*	65
	150.0	6.50 ± 0.56*	88
Morphine	10.0	0.00*	100
Methanol extract	37.5	56.00 ± 1.37	7
	75.0	33.33 ± 2.36*	45
	150.0	19.83 ± 1.66*	67

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 ($p < 0.05$)

two fractions i.e. hexane and methanol fractions. Both fractions and reference drugs were tested for their analgesic, antipyretic and anti-inflammatory activities.

2. Effect of hexane fraction, methanol fraction, aspirin and morphine on acetic acid-induced writhing response in mice

As shown in Table 2 and Fig. 8 morphine at a dose of 10 mg/kg completely inhibited the writhing response. Aspirin at doses of 37.5, 75 and 150 mg/kg also exerted significant inhibition on the number of writhes induced in mice by acetic acid with the percentage of inhibition of 17, 64, 91, respectively. The inhibitory effect of aspirin was found to be dose-related (r=0.9880). The effective inhibitory dose at 50% (ED_{50}) of aspirin determined from its dose-response curve (Fig. 9) was found to be 65.31 mg/kg. Hexane fraction was found to possess profound inhibitory activity on writhing response. At doses of 37.5, 75, and 150 mg/kg, the hexane fraction showed reduction of writhes with the percentage of 26, 69, 93, respectively. The inhibitory effect on the writhing response of the hexane extract was dose-related with the r value of 0.9869. The ED_{50} value of the hexane fraction determined from its dose-response curve (Fig. 9) that was found to be 57.68 mg/kg.

Table 2. Effect of aspirin, morphine as well as hexane and methanol fractions of *D. variegata* on acetic-acid induced writhing response in mice

Group	Dose (mg/kg)	No. of writhes	Inhibition (%)
Control	-	58.33 ± 1.82	-
Aspirin	37.5	47.66 ± 2.99*	17
	75.0	21.33 ± 2.83*	64
	150.0	5.33 ± 0.76*	91
Morphine	10.0	0.00*	100
Hexane fraction	37.5	42.83 ± 1.07*	26
	75.0	17.83 ± 2.70*	69
	150.0	3.66 ± 0.88*	93
Methanol fraction	150.0	55.50 ± 1.73	3

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 ($p < 0.05$)

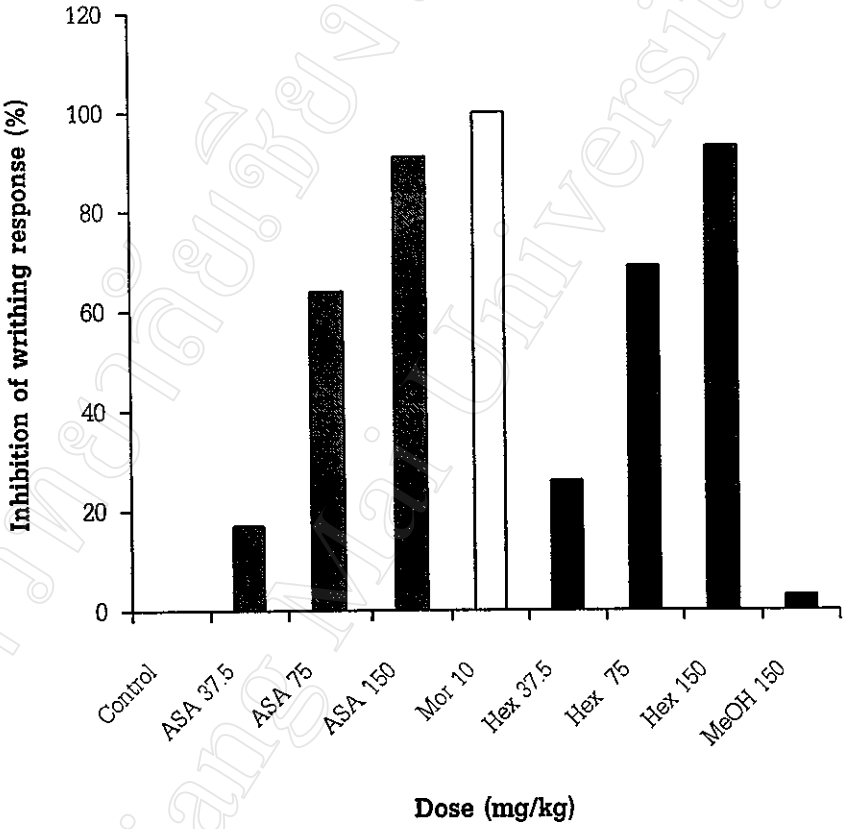

Fig. 8 Histogram of the inhibitory effect of aspirin (■ASA), morphine (□Mor) as well as hexane (■ Hex) and methanol (■ MeOH) fractions of *D. variegata* on acetic acid-induced writhing response in mice. Test drugs were given intraperitoneally 30 min before acetic acid injection.

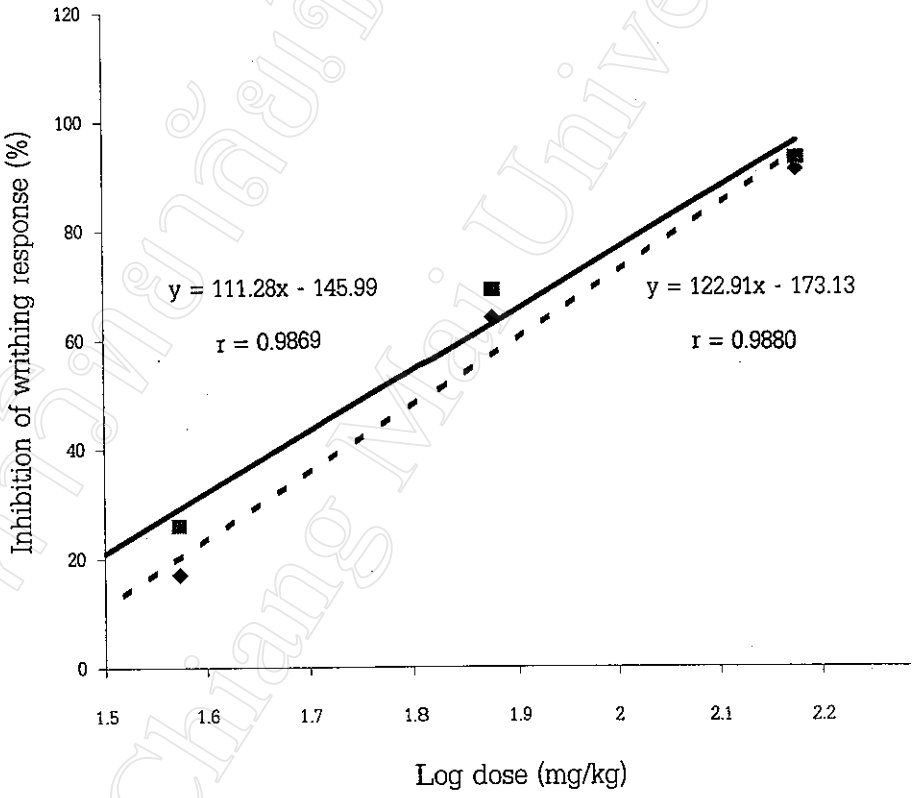

Fig. 9 Log dose-response regression line of aspirin (◆----◆) and hexane fraction (■——■) of *D. variegata* on the acetic acid-induced writhing response in mice.

Methanol fraction, on the contrary, possessed no analgesic effect in this model. The dose of 150 mg/kg, the highest dose used elicited only nonsignificant inhibition on the number of writhes with the percentage inhibition of 3 (Table 2).

3. Effect of hexane fraction, methanol fraction, aspirin and morphine on the rat tail-flick reflex

The tail-flick is a spinal reflex, which is modulated by supraspinal inhibitory mechanisms. Pain on the rat's tail was induced by applying the heat from an infrared source (100 watt bulb). The rat flicked its tail when it felt pain. A 10 s cut-off time was set in order to avoid any damage to the animals. Animals with a control reaction time outside the range of 2-4 s were rejected.

The inhibitory effects of hexane fraction, methanol fraction, aspirin and morphine on the tail-flick reflex in rats are shown in Table 3. Morphine, an opioid and a centrally acting analgesic drug, completely inhibited the tail-flick response (100%) when an intraperitoneal dose of 10 mg/kg was used. Aspirin, at a dose of 150 mg/kg possessed slight analgesic effect of 16% in this test model. Similarly, hexane fraction at the dose of 150 mg/kg exhibited only slight analgesic effect of 21% on the tail-flick reflex. Methanol fraction at the same dose

Table 3. Effect of aspirin, morphine as well as hexane and methanol fractions of *D. variegata* on the tail-flick test in rats

Group	Dose (mg/kg)	T_c (s)	T_r (s)	Inhibition (%)
Control	-	2.77 ± 0.16	2.97 ± 0.20	-
Aspirin	150	2.63 ± 0.19	3.83 ± 0.22*	16
Morphine	10	2.93 ± 0.26	10.00 ± 0.00*	100
Hexane fraction	150	2.63 ± 0.18	4.20 ± 0.32*	21
Methanol fraction	150	3.21 ± 0.21	3.45 ± 2.20	4

Test drugs were administered intraperitoneally 30 min before experiment.

Values were expressed as Mean ± S.E.M. (n = 6)

* Significantly different from T_c ($p < 0.05$)

T_c = control reaction time; T_r = reaction time after injection of test drugs

(150 mg/kg) did not show any analgesic effect in this test model.

4. Effect of hexane fraction, methanol fraction, aspirin and morphine on the formalin test in mice

The analgesic test using formalin-induced pain at the right dorsal hindpaw of mice was performed using the intensive licking time as a criterion for indicating pain. The results obtained at the early phase as shown in Table 4 showed that morphine at a dose of 10 mg/kg completely abolished licking. Aspirin at doses of 75 and 150 mg/kg could slightly inhibit the licking response with the percentage of 24 and 38, respectively. Hexane fraction at doses of 75 and 150 mg/kg, similarly to aspirin, possessed only mild inhibitory effect on licking with the percentage of 16 and 38, respectively. At the dose of 37.5 mg/kg, Aspirin and hexane fraction did not show inhibitory activity on the licking response. Methanol fraction at the highest dose (150 mg/kg), on the contrary, could not inhibit the licking response in this test model.

Results obtained at the late phase of formalin test (Table 5) revealed the marked analgesic effect of morphine as shown by no licking at all after a morphine dose of 10 mg/kg. Aspirin

Table 4. Effect of aspirin, morphine as well as hexane and methanol fractions of *D. variegata* on the early phase of formalin test in mice

Group	Dose (mg/kg)	Licking time[a] (s)	Inhibition (%)
Control	-	104.33 ± 7.69	-
Aspirin	37.5	98.33 ± 3.54	6
	75	79.17 ± 4.47*	24
	150	65.17 ± 2.00*	38
Morphine	10	0.00*	100
Hexane fraction	37.5	97.33 ± 3.77	7
	75	86.83 ± 3.41*	16
	150	65.17 ± 2.94*	38
Methanol fraction	150	105.17 ± 5.04	0

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 5. Effect of aspirin, morphine as well as hexane and methanol fractions from *D. variegata* on the late phase of the formalin test in mice

Group	Dose (mg/kg)	Licking time[a] (s)	Inhibition (%)
Control	-	115.33 ± 10.20	-
Aspirin	37.5	73.17 ± 1.19*	37
	75	50.17 ± 7.02*	57
	150	31.67 ± 3.79*	72
Morphine	10	0.00*	100
Hexane fraction	37.5	73.67 ± 2.80*	36
	75	46.00 ± 2.48*	60
	150	31.33 ± 3.58*	73
Methanol fraction	150	110.50 ± 5.78	3

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 0-10 min after formalin injection

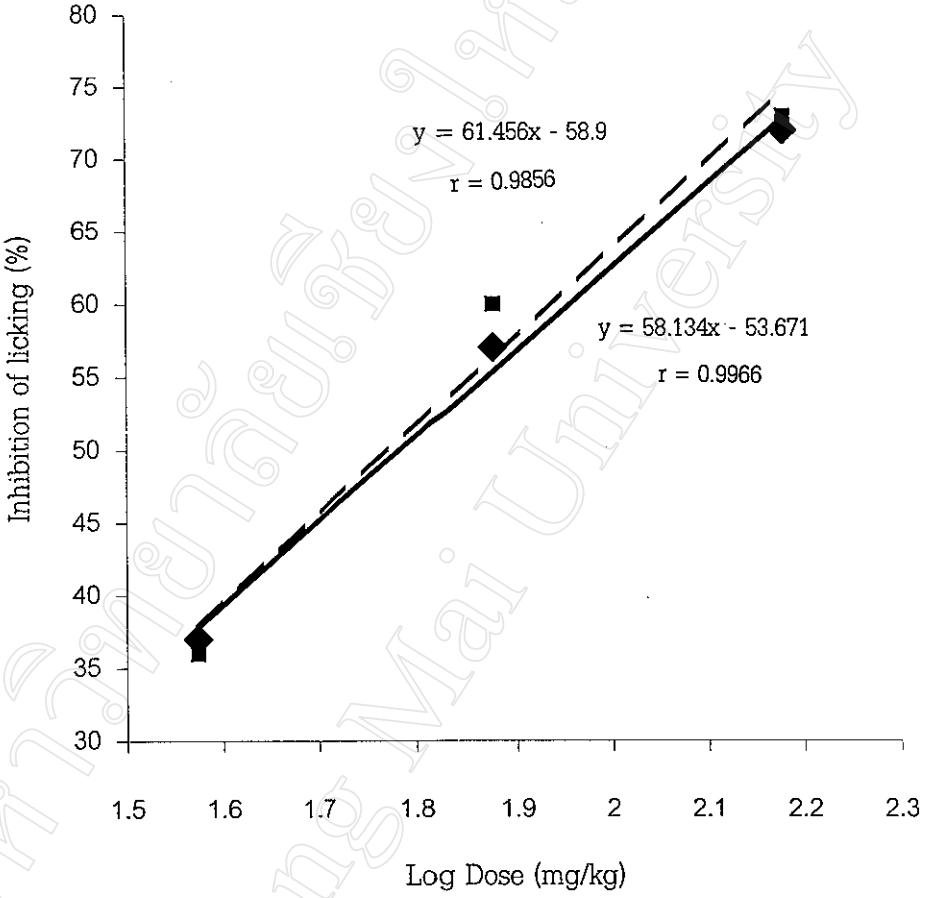

Fig. 10 Log dose-response regression line of aspirin (◆--◆) and hexane fraction (■——■) of *D. variegata* on the formalin test in mice (late phase)

at doses of 37.5, 75 and 150 mg/kg also showed inhibitory effect on licking with the percentage of 37, 57, 72, respectively. The ED_{50} value of aspirin determined from its dose-response curve (Fig. 10) was found to be 60.72 mg/kg. According to the positive correlation coefficient (r) value (0.9856), it was suggested that the inhibitory effect of aspirin on the late phase of formalin test was dose-related. Similar to aspirin hexane fraction at doses of 37.5, 75, and 150 mg/kg exhibited moderate analgesic effect on this test model with the percentage of inhibition on licking of 36, 60, 73, respectively (Table 5). The ED_{50} value of hexane fraction determined from its dose-response curve (Fig. 10) that was found to be 59.14 mg/kg. The positive correlation coefficient (r) value (0.9966) obtained suggested the dose-related inhibitory effect of the hexane fraction on the late phase of formalin test. In contrast to the hexane fraction, the methanol fraction, even at the highest dose (150 mg/kg) possessed no analgesic effect on this test model (Table 5).

5. Effect of hexane fraction, methanol fraction, aspirin and morphine on ear edema in rats induced by ethyl phenylpropiolate (EPP) and arachidonic acid (AA)

Results obtained from the EPP-induced rat ear-edema are summarized in Table 6. Phenylbutazone, a potent nonsteroidal anti-inflammatory drug, at the dose of 1 mg/ear inhibited the edema formation significantly. The highest inhibitory effect (55%) of phenylbutazone was obviously seen by the first measurement at 15 min after application of EPP. The percent of inhibition slightly decreased to 52, 52 and 51 when measurement was made at 30, 60 and 120 min after EPP. Hexane fraction at the dose of 4 mg/ear significantly inhibited the ear edema formation with percent of inhibition of 58, 60, 58 and 55 at 15, 30, 60 and 120 minutes, respectively, after the edema induction. Methanol fraction at the dose of 4 mg/ear could not inhibit the edema formation induced by EPP.

Results obtained from the rat ear-edema induced by AA are demonstrated in Table 7. Phenylbutazone, a cyclooxygenase inhibitor, at the dose of 1 mg/kg ear, could inhibit the edema formation significantly only at 15 min after AA application. When measurement of ear thickness was made at 30, 60 and 120 min after edema induction, it was found that phenylbutazone could not prevent the edema formation induced

by AA. Phenidone, a dual inhibitor of cyclooxygenase and lipoxygenase at the dose of 2 mg/ear inhibited edema formation significantly with the percent inhibition of 39, 47, 56 and 55 at 15, 30, 60 and 120 min after AA. Similarly, the hexane fraction at the dose of 4 mg/ear elicited significantly inhibitory effect on the ear edema formation with the percentage of 26, 41, 52 and 50 at 15, 30, 60 and 120 min after the edema induction. The methanol fraction, on the contrary, could not exert any inhibitory effect on the edema formation induced by AA.

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Table 6. Effect of topical application of phenylbutazone, hexane and methanol fractions of *D. variegata* on ethyl phenylpropiolate-induced ear edema in rats

Group	Dose	Edema thickness (μm)				inhibition (%)			
	(mg/ear)	15 min	30 min	1 h	2 h	15 min	30 min	1 h	2 h
Control	-	97 ± 8	210 ± 7	213 ± 13	190 ± 11	-	-	-	-
Phenylbutazone	1	44 ± 4*	100 ± 4*	103 ± 6*	94 ± 3*	55	52	52	51
Hexane fraction	4	40 ± 7*	85 ± 13*	90 ± 9*	85 ± 6*	58	60	58	55
Methanol fraction	4	88 ± 10	217 ± 8	200 ± 14	180 ± 15	9	0	6	5

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

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

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Table 7. Effect of topical application of phenylbutazone, phenidone as well as hexane and methanol fractions of *D. variegata* on arachidonic acid-induced ear edema in rats

Group	Dose	Edema thickness (μm)				% inhibition			
	(mg/ear)	15 min	30 min	1 h	2 h	15 min	30 min	1 h	2 h
Control	-	77 ± 11	207 ± 8	273 ± 10	247 ± 4	-	-	-	-
Phenylbutazone	1	58 ± 15*	206 ± 18	250 ± 10	243 ± 10	25	0	8	2
Phenidone	2	47 ± 11*	110 ± 7*	120 ± 7*	110 ± 4*	39	47	56	55
Hexane fraction	4	57 ± 6*	123 ± 12*	130 ± 9*	123 ± 6*	26	41	52	50
Methanol fraction	4	67 ± 21	193 ± 29	207 ± 12	210 ± 11	13	7	3	0

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

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

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6. Effect of aspirin, hexane fraction and methanol fraction on yeast-induced hyperthermia in rats

The hyperthermia was induced in rats by subcutaneous injection of 20% brewers yeast suspension. About 18 h after the injection the animals developed fever. The test agents were intraperitoneally administered into animals, which showed an increase in the body temperature of not less than 1 °C. The rectal temperature was measured every 30 min for 2 hr after drug administration.

As shown in Table 8 and Fig. 11, 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. Similar results were observed with the hexane fraction. It was also found that the hexane fraction seemed to possess the same antipyretic activity as that of aspirin, since a dose of 150 mg/kg of hexane fraction could reduce the rectal temperature of the rats to 38.43 ± 0.18, 37.87 ± 0.16, 37.43 ± 0.17 and 37.30 ± 0.19 when measurement was made 30, 60, 90 and 120 minutes, respectively, after drug administration. Aspirin, at the dose of 150 mg/kg reduced the hyperthermia to 38.37 ± 0.21, 38.09 ± 0.26, 37.67 ± 0.24 and 37.58 ± 0.24 at 30, 60, 90 and 120 minutes, respectively, after drug administration. The methanol

fraction at the dose of 150 mg/kg did not possess any antipyretic effect.

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Table 8. Effect of aspirin, hexane and methanol fractions of *D. variegata* on yeast-induced hyperthermia in rats

Group	Dose	Rectal temperature (°C)					
		Initial	18 h after yeast inj.	Time after medication (min)			
	(mg/kg)		0	30	60	90	120
Control	-	38.30 ± 0.07	39.35 ± 0.06	39.30 ± 0.09	39.23 ± 0.07	39.20 ± 0.08	39.15 ± 0.08
Aspirin	150	38.30 ± 0.10	39.35 ± 0.06	38.37 ± 0.21*	38.09 ± 0.26*	37.67 ± 0.24*	37.58 ± 0.24*
Hexane fraction	150	38.28 ± 0.08	39.37 ± 0.12	38.43 ± 0.18*	37.87 ± 0.16*	37.43 ± 0.17*	37.30 ± 0.19*
Methanol fraction	150	38.25 ± 0.06	39.20 ± 0.06	39.17 ± 0.03	39.07 ± 0.65	39.05 ± 0.03	39.00 ± 0.03

Drugs were given (i.p.) 18 h after yeast injection.

Valued were expressed as Mean ± S.E.M. (n = 6)

* Significantly different from the rectal temperature after yeast injection 18 h ($p < 0.05$)

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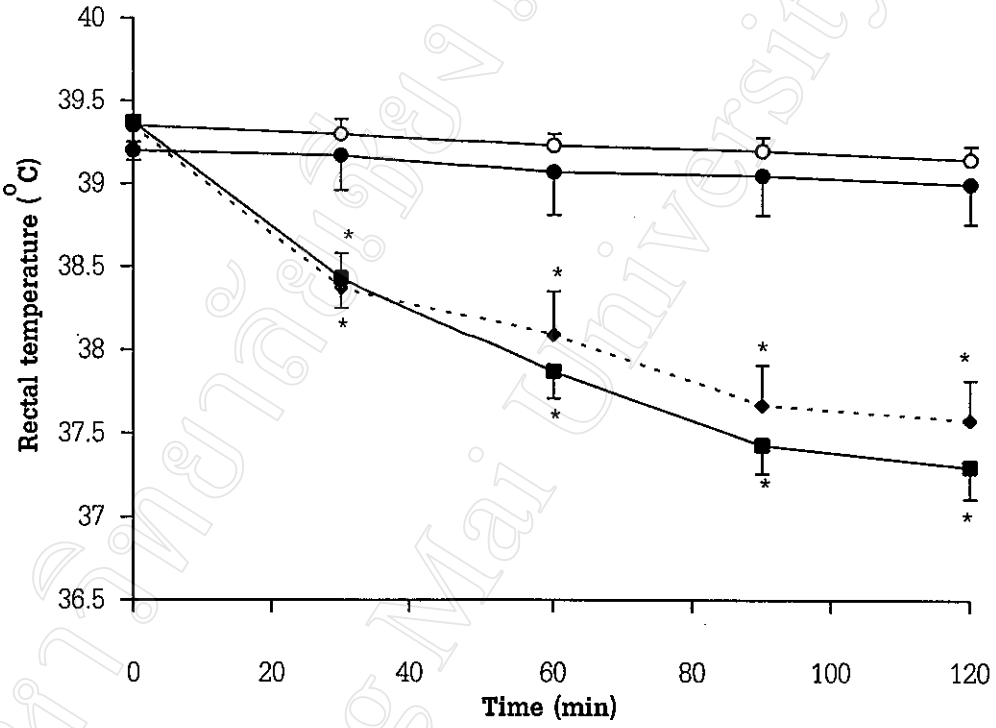

Fig. 11. Effect of control (○——○), aspirin (◆- - - - - - - ◆), hexane (■——■)and methanol (●——●) fractions of *D. variegata* on yeast-induced hyperthermia in rats. Test drugs (150 mg/kg) were given intraperitoneally 18 h after yeast injection.

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