CHAPTER 4

RESULTS

1. Acute toxicity

No death was found during the treatment period in either the control or treated groups given the methanol extract of *Kaempferia galanga* up to 5 g/kg orally in male mice and rats. The animals did not show any changes in general behaviors or signs of toxicities or other abnornal physiological activities during the observation period.

2. Analgesic activity

1.1 Writhing test

The effects of methanol extract of *K. galanga* on 0.6% acetic acid–induced writhing in mice were summarized in Table 7 and Figure 20. Oral administration of the extract at doses of 50, 100 and 200 mg/kg dose dependently decreased (p<0.01) the number of writhings and stretchings (29.6 ± 0.95, 20.9 ± 1.53 and 15.2 ± 1.13, respectively) when compared to the control (51.7 ± 2.54) with the percentage of inhibition being 42.75, 59.57 and 70.6, respectively. The standard drug aspirin at a dose of 100 mg/kg significantly decreased the number of writhings produced by 0.6% acetic acid intraperitoneally injected when compared to the control and the percentage of inhibition was 51.06 (25.3±1.25 vs 51.7±2.54; p<0.01). *K. galanga* (200 mg/kg) significant inhibited the number of writhings with an inhibition of 42.75% while aspirin (100 mg/kg) had an inhibition of 51%. The result indicated that *K. galanga* (200 mg/kg) was more potent than aspirin (100 mg/kg).

Table 7. Effects of the methanol extract of *Kaempferia galanga* (MEKG) and aspirin on acetic acid-induced writhing in mice.

Treatment	Dose(mg/kg, p.o.)	Number of writhings	Inhibition (%)
Cosolvent		51.7±2.54	0
Aspirin	100	25.3±1.25*	51.06
MEKG	50	29.6±0.95*	42.75
MEKG	100	20.9±1.53*	59.57
MEKG	200	15.2±1.13*	70.60

Values are presented as mean ± S.E.M. (N=10)

Thirty minutes after the oral administration of the test agents, mice were intraperitoneally injected with 0.6% (V/V) acetic acid.

Number of writhings measured in a 20 minute period after intraperitoneally injecting with 0.6% (v/v) acetic acid.

* $p < 0.01$, significantly different compared with control (Bonferroni's test).

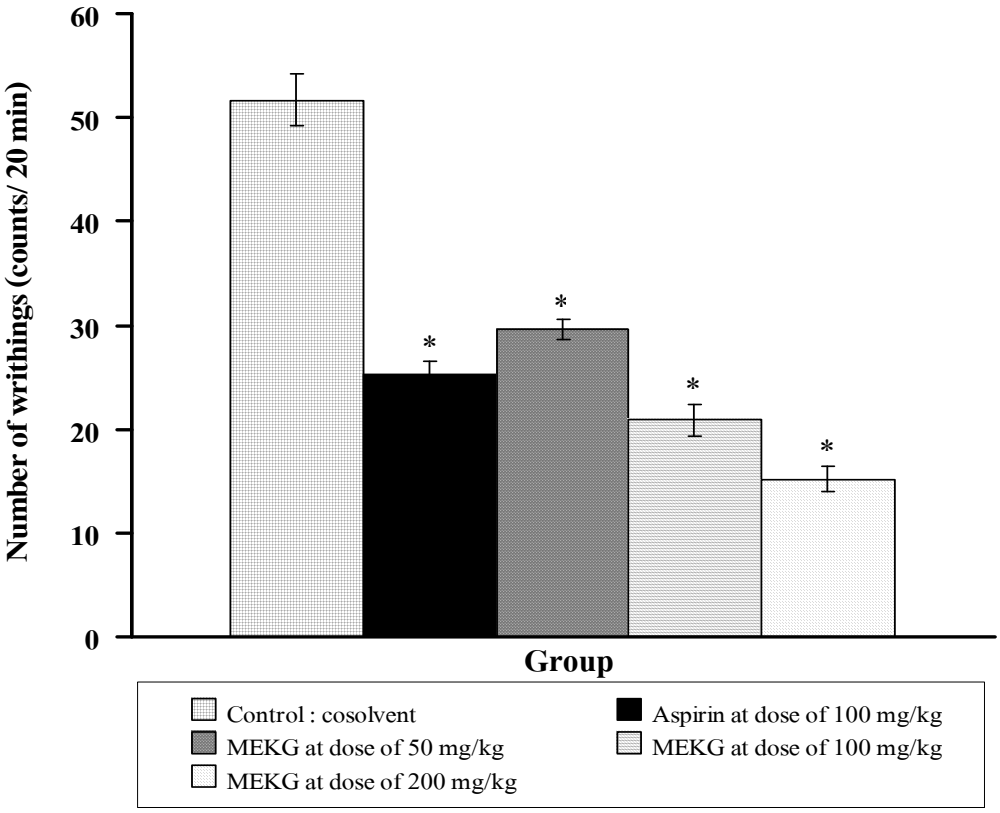

Figure 20. Effects of the methanol extract of *Kaempferia galanga* (MEKG) and aspirin on acetic acid-induced writhing in mice.

$^{*}p < 0.01$, significantly different compared to the control (Bonferroni's test).

1.2 Formalin test

The results (Table 8 and Figure 21) show that the times spent in licking the paw after standard drug morphine (5 mg/kg, s.c.) and aspirin (100 mg/kg, p.o.) were given 30 minutes before the injection of 20 µL of 2.5% formalin at the right hind paw of the mice were significantly decreased both in the early and late phase compared to the control (p<0.01). The total licking time (second) at the hind paw in the early phase (0-5 minutes) after treatment with morphine and aspirin were 25.61 ± 1.36 and 41.72 ± 2.24, respectively compared to the control (77.17 ± 3.62), while in the late phase (15-30 minutes) were 17.63 ± 1.01 and 76.94 ± 2.71, respectively when compared to the control (94.03 ± 3.22). The percentage of inhibition after treatment with morphine and aspirin in late phase were 81.25 and 18.18, respectively. In the early phase, *K. galanga* at doses of 50, 100 and 200 mg/kg significantly decreased the total licking time (p<0.01) produced by 2.5% formalin (54.97±2.58, 52.04±2.42 and 35.90±2.97), respectively when compared to the control (77.17±3.62) with the percentage of inhibition being 28.77, 32.56 and 53.48, respectively. In the late phase (15-30 minute), *K. galanga* at doses of 50, 100 and 200 mg/kg significantly reduced the licking time when compared to the control (29.21±1.93, 19.97±2.06 and 20.22±0.96, respectively vs 94.03±3.22; p<0.01) with the percentage of inhibition being 68.94, 78.76 and 78.50, respectively. The results showed that aspirin slightly inhibited the licking response in the late phase and indicated that all doses of the extract used, significantly decreased the time spent in licking both in early and late phase.

Table 8. Effects of the methanol extract of *Kaempferia galanga* (MEKG), morphine and aspirin on 2.5% formalin-induced paw licking in mice.

Treatment	Dose (mg/kg, p.o.)	Licking of the hind paw (sec.)			
		Early phase (0-5 min)	Inhibition (%)	Late phase (15-30 min)	Inhibition (%)
Cosolvent		77.17±3.62	0	94.03±3.22	0
Morphine	5, s.c.	25.61±1.36*	66.83	17.63±1.01*	81.25
Aspirin	100	41.72±2.24*	45.94	76.94±2.71*	18.18
MEKG	50	54.97±2.58*	28.77	29.21±1.93*	68.94
MEKG	100	52.04±2.42*	32.56	19.97±2.06*	78.76
MEKG	200	35.90±2.97*	53.48	20.22±0.96*	78.50

Values are presented as mean ± S.E.M. (N=10)

Thirty minutes after the oral administration of the test agents, mice were subcutaneously injected in the hind paw with 2.5% (v/v) formalin.

Fifteen minutes after morphine, mice were subcutaneously injected in the hind paw with 2.5% (v/v) formalin.

*p< 0.01, significantly different compared to the control (Bonferroni's test).

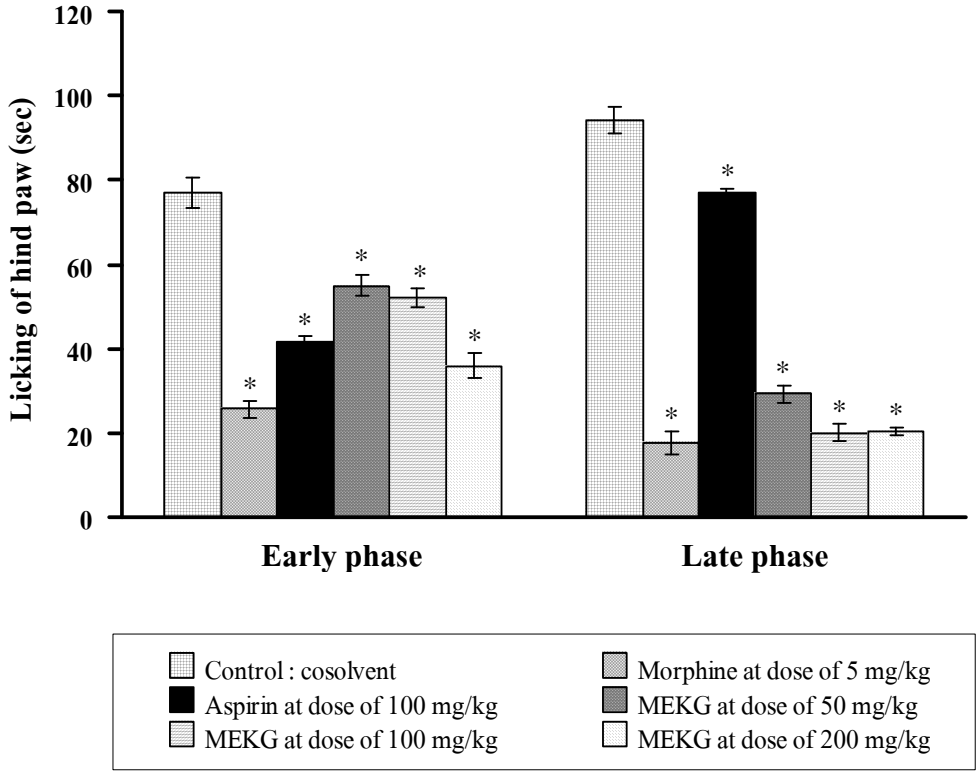

Figure 21. Effects of the methanol extract of *Kaempferia galanga* (MEKG), morphine and aspirin on 2.5% formalin-induced paw licking in mice.

$^{*}p < 0.01$, significantly different compared to the control (Bonferroni's test).

1.3 Hot plate test

The results showed that morphine at the dose of 5 mg/kg s.c. markedly increased the pain latency at all the time intervals measured (30, 45, 60, 75 and 90 minutes; $p<0.01$) after administration. *K. galanga* at doses of 50 and 100 mg/kg significantly increased latency of nociceptive response at 90 minutes (10.77±0.37; $p<0.05$ and 11.45±0.30; $p<0.01$, respectively) when compared to the control (8.74±0.37), while *K. galanga* at the dose of 200 mg/kg significantly increased pain latency in mice at 45 ($p<0.05$), 60 ($p<0.01$), 75 ($p<0.01$) and 90 ($p<0.01$) minutes after the administration compared to the control. The results indicated that *K. galanga* at the dose of 50 mg/kg slightly significantly delayed the pain responses in mice. *K. galanga* at the dose of 200 mg/kg exhibited more potent effects to delay the latency of nociceptive response than *K. galanga* at doses of 50 and 100 mg/kg. The results were summarized in Table 9 and Figure 22.

The antagonistic action of naloxone on effects of morphine or *K. galanga* (200 mg/kg) on the latency of nociceptive response was also investigated. The results showed that naloxone (2 mg/kg, i.p.) given before morphine (5 mg/kg, s.c) also antagonized the effect of morphine at 45, 60, 75 and 90 minutes ($p<0.01$) compared to the morphine administered alone. Furthermore, naloxone (2 mg/kg, i.p.) given before *K. galanga* (200 mg/kg, p.o.) significantly decreased the latency of nociceptive response of the extract at 75 and 90 minutes ($p<0.01$) when compared with *K. galanga* at the dose of 200 mg/kg given alone (Table 10, Figure 23).

Table 9. Effects of the methanol extract of *Kaempferia galanga* (MEKG) and morphine on heat-induced pain in mice.

Treatment	Dose	Latency of nociceptive response (sec.)				
	(mg/kg, p.o.)	30 min	45 min	60 min	75 min	90 min
Cosolvent		8.56±0.22	8.71±0.28	9.19±0.21	9.42±0.23	8.74±0.37
Morphine	5, s.c.	10.61±0.39**	14.37±0.68**	17.74±0.59**	17.49±0.48**	14.71±0.73**
MEKG	50	8.84±0.25	9.10±0.33	9.35±0.27	10.74±0.42	10.77±0.37*
MEKG	100	9.48±0.42	9.77±0.22	10.15±0.14	10.36±0.30	11.45±0.30**
MEKG	200	9.71±0.34	10.57±0.27*	12.43±0.43**	13.38±0.37**	12.87±0.41**

Values are presented as mean ± S.E.M. (N=10)

Latency of nociceptive response was initially measured 30 min after the oral administration of MEKG (or 15 min after the morphine injection s.c.), then every 15 min up to a 90-minute period.

*p <0.05, **p< 0.01, significantly different compared to the control (Bonferroni's test).

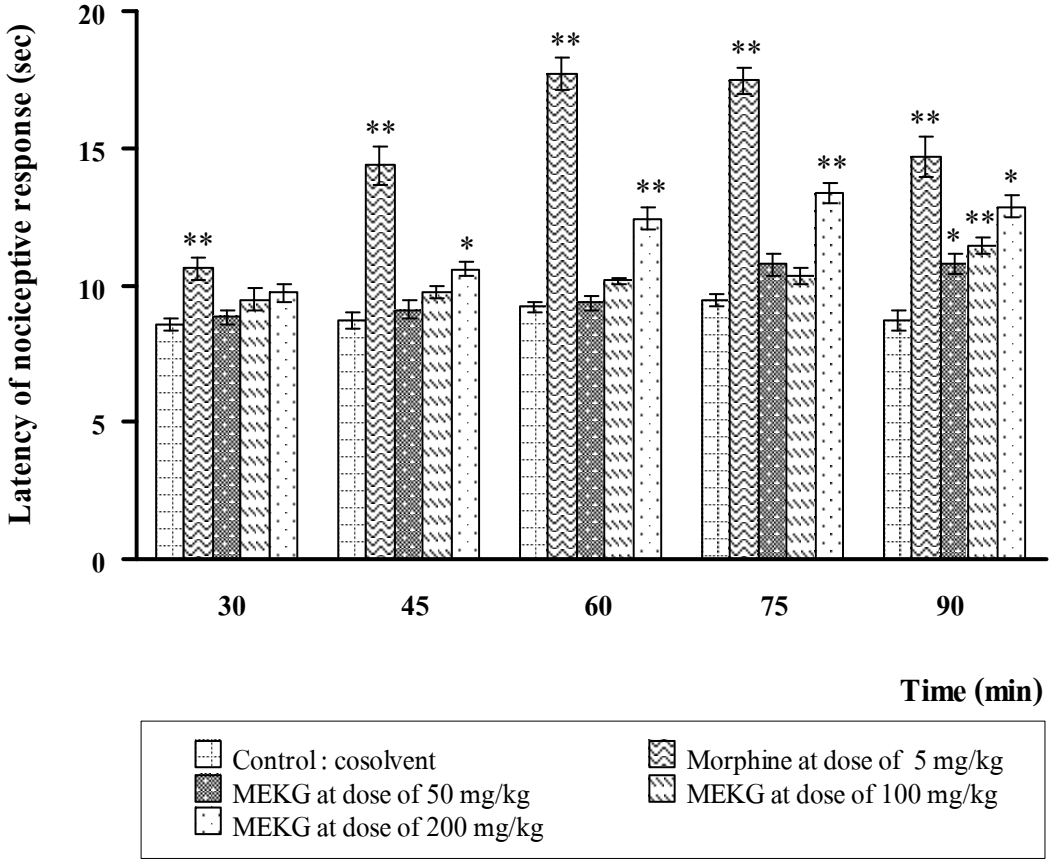

Figure 22. Effects of the methanol extract of *Kaempferia galanga* (MEKG) (50, 100 and 200 mg/kg) and morphine, s.c. on heat-induced pain in mice.

$^{*}p < 0.05$, $^{**}p < 0.01$, significantly different compared to the control (Bonferroni's test)

Table 10. Antagonistic effects of naloxone on morphine and methanol extract of *Kaempferia galanga* (MEKG) on heat-induced pain in mice.

Treatment	Dose	Latency of nociceptive response (sec.)				
	(mg/kg)	30 min	45 min	60 min	75 min	90 min
Cosolvent		8.56±0.22	8.71±0.28	9.19±0.21	9.42±0.23	8.74±0.37
Morphine	5, s.c.	10.61±0.39	14.37±0.68	17.74±0.59	17.49±0.48	14.71±0.73
Naloxone	2, i.p.					
+ Morphine	5, s.c.	9.57±0.49	9.61±0.40[a]	9.63±0.36[a]	10.11±0.40[a]	10.43±0.51[a]
MEKG	200, p.o.	9.71±0.34	10.57±0.27	12.43±0.43	13.38±0.37	12.87±0.41
Naloxone	2, i.p.					
+ MEKG	200, p.o.	10.28±0.57	10.65±0.45	11.27±0.63	11.22±0.54[#]	10.13±0.62[#]

Values are presented as mean ± S.E.M. (N=10)

Latency of nociceptive response was initially measured 30 min after the oral administration of MEKG (or 15 min after the morphine injection s.c.), then every 15 min up to a 90 minute period. Naloxone (2 mg/kg) was intraperitoneal injected 10 min before test agents were administered in mice.

[a] p <0.01, significantly different compared to the morphine (Independent t-test).

[#] p< 0.01, significantly different compared to the MEKG at dose of 200 mg/kg (Independent t-test).

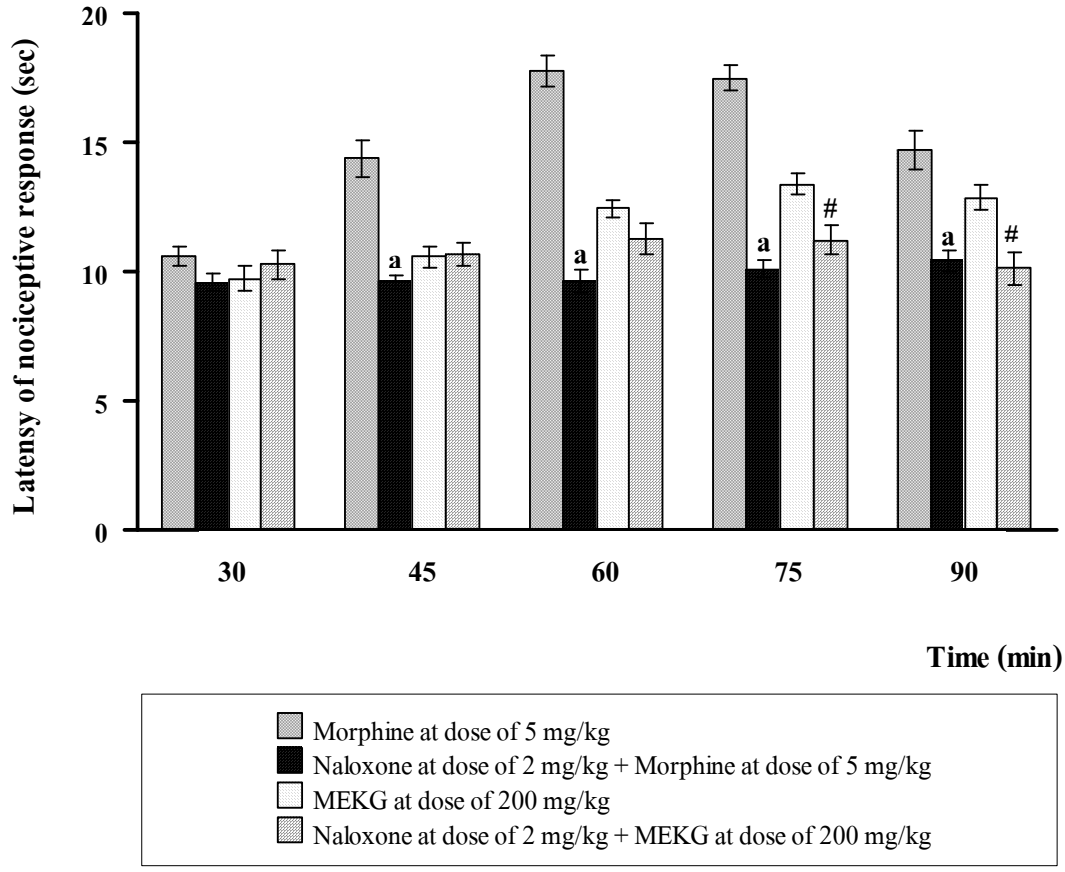

Figure 23. Antagonistic effects of naloxone (2 mg/kg, i.p.) on morphine (5 mg/kg, s.c.) and methanol extract of *Kaempferia galanga* (MEKG) at the dose of 200 mg/kg orally on heat-induced pain in mice.

[a] $p < 0.01$, significantly different compared to the morphine (Independent *t*-test).

[#] $p < 0.01$, significantly different compared to the MEKG at dose of 200 mg/kg (Independent *t*-test).

1.4 Tail flick test

Administration of the extract (50, 100 and 200 mg/kg, p.o.) and morphine (5 mg/kg, s.c.) significantly increased the reaction time to the nociceptive responses. The antinociceptive effect of the extract at doses of 50, 100 and 200 mg/kg, p.o. began at the time 45 ($p<0.05$), 75 ($p<0.05$) and 45 ($p<0.01$) minutes, respectively after the oral administration, when compared to the control, and persisted until the time at 75 minutes. Morphine (5 mg/kg, s.c.), a centrally acting analgesic drug , significantly increased ($p<0.01$) the tail flick latency at all the time intervals measured in the tail flick test in rats, but its antinociceptive effect still remained for 90 minutes.

In antagonistic studies of naloxone, the results showed that naloxone (2 mg/kg, i.p.) completely antagonized the effect of morphine (5 mg/kg, s.c.) on the latency of nociceptive response at all the time intervals measured ($p<0.01$). Furthermore, naloxone at the dose of 2 mg/kg, i.p. given before *K. galanga* at the dose of 200 mg/kg significantly decreased the latency of nociceptive response produced by the extract in the tail flick test at 30 ($p<0.05$), 45 ($p<0.01$), 60 ($p<0.01$), 75 ($p<0.01$) and 90 ($p<0.05$) minutes when compared to the *K. galanga* at the dose of 200 mg/kg (Table 12, Figure 25).

Table 11. Effects of the methanol extract of *Kaempferia galanga* (MEKG) and morphine on nociceptive responses in the tail flick tests in rats.

Treatment	Dose	Latency of nociceptive response (sec.)				
	(mg/kg, p.o.)	30 min	45 min	60 min	75 min	90 min
Cosolvent		1.31±0.03	1.27±0.05	1.26±0.04	1.21±0. 10	1.24±0.03
Morphine	5, s.c.	2.43±0.05**	3.55±0.05**	4.39±0.06**	3.58±0.07**	3.23±0.05**
MEKG	50	1.32±0.03	1.42±0.02*	1.46±0.02*	1.41±0.02**	1.34±0.02
MEKG	100	1.33±0.03	1.33±0.02	1.37±0.02	1.37±0.02*	1.31±0.02
MEKG	200	1.39±0.03	1.46±0.02**	1.42±0.02*	1.41±0.02**	1.32±0.02

Values are presented as mean ± S.E.M. (N=10)

Latency of nociceptive response was initially measured 30 min after the oral administration of MEKG (or 15 min after the morphine injection, s.c.), then every 15 min up to 90-minute period.

*p <0.05, **p< 0.01, significantly different compared to the control (Bonferroni's test).

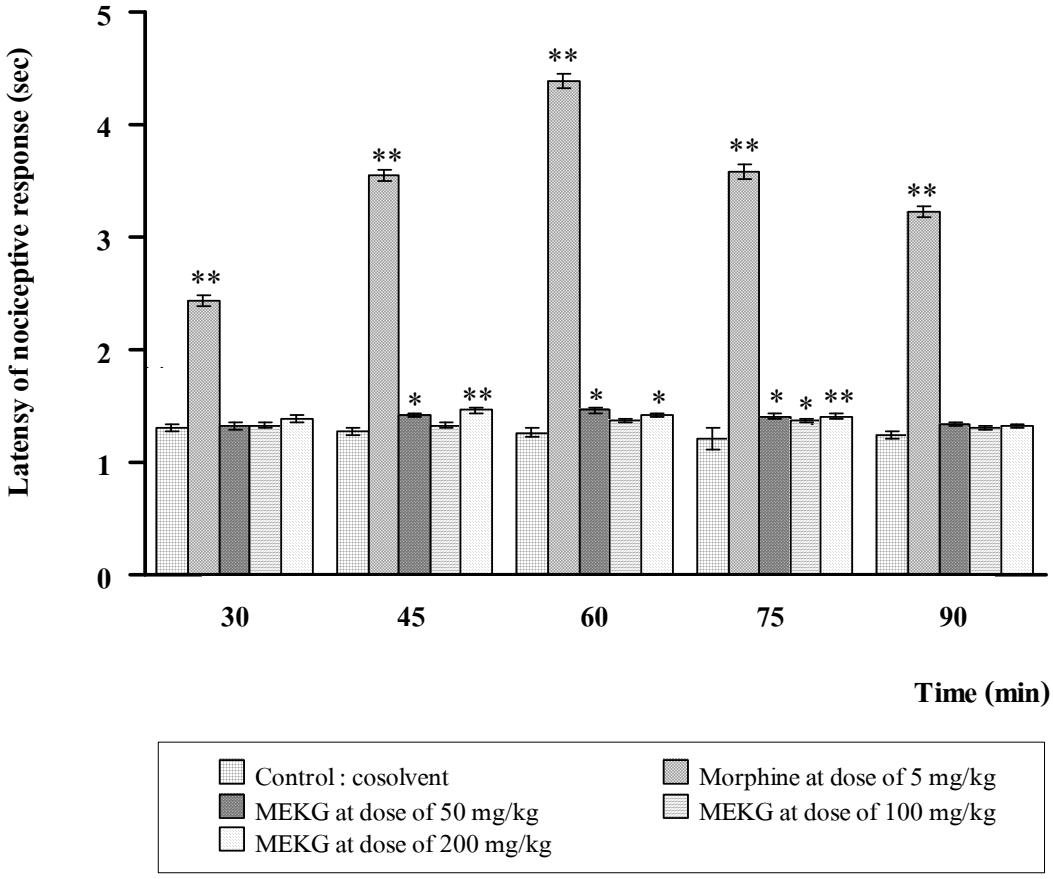

Figure 24. Effects of the methanol extract of *Kaempferia galanga* (MEKG) (50, 100 and 200 mg/kg) and morphine on nociceptive responses in the tail flick tests in rats.

$^{*}p < 0.05$, $^{**}p < 0.01$, significantly different compared to the control (Bonferroni's test).

Table 12. Antagonistic effects of naloxone on morphine and methanol extract of *Kaempferia galanga* (MEKG) on nociceptive responses in the tail flick tests in rats.

Treatment	Dose	Latency of nociceptive response (s)				
	(mg/kg)	30 min	45 min	60 min	75 min	90 min
Cosolvent		1.31±0.03	1.27±0.05	1.26±0.04	1.21±0. 10	1.24±0.03
Morphine	5, s.c.	2.43±0.05**	3.55±0.05**	4.39±0.06**	3.58±0.07**	3.23±0.05**
Naloxone	2, i.p.					
+ Morphine	5, s.c.	1.59±0.05[a]	1.50±0.03[a]	1.42±0.02[a]	1.41±0.02[a]	1.35±0.04[a]
MEKG	200, p.o.	1.39±0.03	1.46±0.02	1.42±0.02	1.41±0.02	1.32±0.02
Naloxone	2, i.p.					
+ MEKG	200, p.o.	1.28±0.03#	1.29±0.03##	1.28±0.04##	1.29±0.02##	1.26±0.02#

Values are presented as mean ± S.E.M. (N=10)

Latency of nociceptive response was initially measured 30 min after oral administration of MEKG (or 15 min after morphine injection s.c.), then every 15 min up to a 90 minute period. Naloxone (2 mg/kg) was intraperitoneal injected 10 min before test agents were administered in the mice.

** $p< 0.01$, significantly different compared to the control (Bonferroni's test).

[a] $p< 0.01$, significantly different compared to the morphine 5 mg/kg (Independent t-test).

# $p< 0.05$, ## $p< 0.01$, significantly different compared to the MEKG 200 mg/kg (Independent t-test).

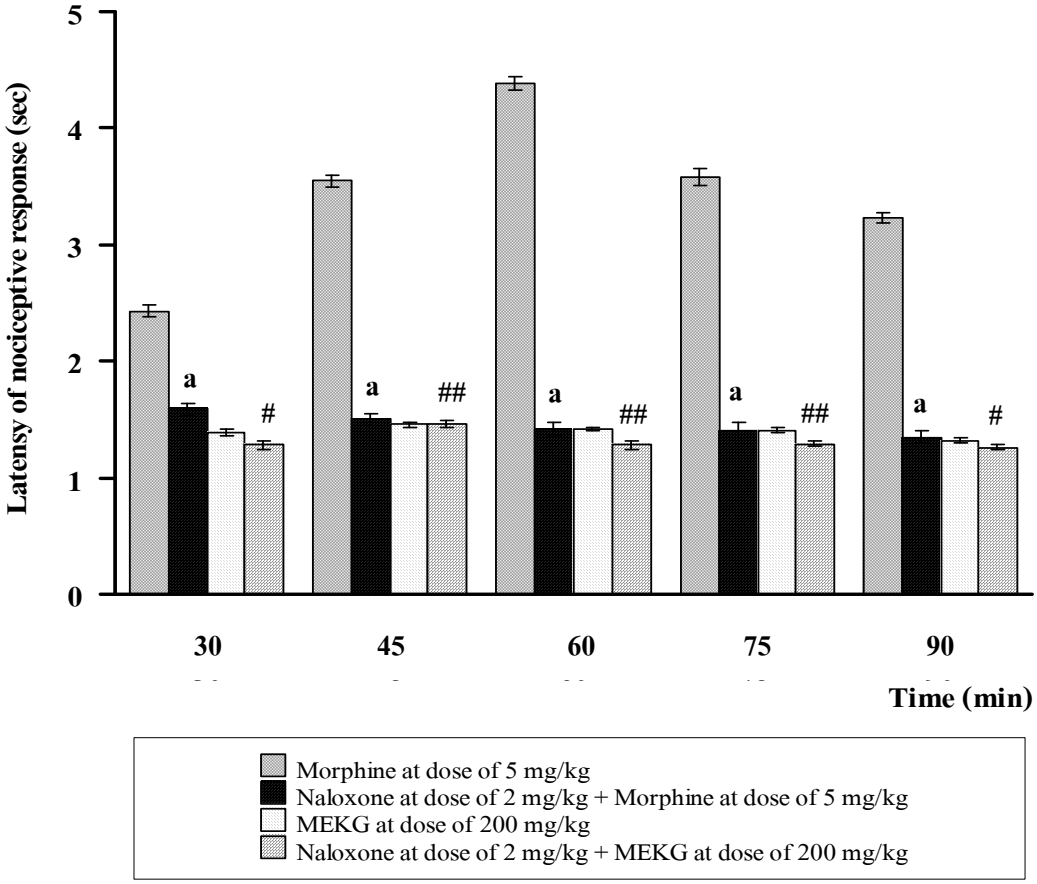

Figure 25. Antagonistic effect of naloxone (2 mg/kg, i.p.) on morphine (5 mg/kg, s.c.) and methanol extract of *Kaempferia galanga* MEKG at dose of 200 mg/kg on nociceptive responses in the tail flick test in rats.

[a]p <0.01, significantly different compared with morphine (Independent t-test).

[#]p <0.05, [##]p< 0.01, significantly different compared with MEKG at dose of 200 mg/kg (Independent t-test).

2. Antipyretic activity

The results of the antipyretic effects of aspirin and *K. galanga* extract were presented in Table 13 and Figure 26. Administration of the brewer's yeast to the rat produced an increase in rectal temperature 19 hours after the yeast injection. Aspirin at the dose of 100 mg/kg, p.o. caused a significant decrease in rectal temperature. The decrease in rectal temperature began at 1 h after the aspirin administration and continued up to 5 h. *K. galanga* at doses of 50, 100 and 200 mg/kg orally did not decrease the rectal temperature induced by the brewer's yeast injection at all the time intervals. The results indicated that *K. galanga* did not exhibit antipyretic activity in the rat induced pyrexia by brewer's yeast injection.

Table 13. Effect of the methanol extract of *Kaempferia galanga* (MEKG) and aspirin on the brewer's yeast induced pyrexia in rats.

Treatment	Dose (mg/kg, p.o.)	Rectal temperature (°c)						
		Before yeast injection	Time after treatment (h)					
			0 h	1 h	2 h	3 h	4 h	5 h
Cosolvent		36.79±0.11	37.53±0.14	37.54±0.09	37.47±0.08	37.44±0.11	37.45±0.08	37.58±0.14
Aspirin	100	36.71±0.07	37.48±0.09	37.05±0.10*	36.97±0.10*	36.85±0.15*	36.74±0.15*	36.80±0.14*
MEKG	50	36.70±0.07	37.29±0.15	37.35±0.10	37.32±0.12	37.02±0.12	36.99±0.11	36.96±0.14
MEKG	100	36.72±0.09	37.55±0.09	37.53±0.15	37.30±0.15	37.24±0.11	36.96±0.17	36.98±0.18
MEKG	200	36.70±0.08	37.59±0.10	37.57±0.13	37.39±0.39	37.21±0.09	37.04±0.23	37.09±0.19

Values are presented as mean ± S.E.M. (N=10).

Rectal temperature measured after the yeast injection 19 h

* $p < 0.05$, significantly different compared to the control (Bonferroni's test).

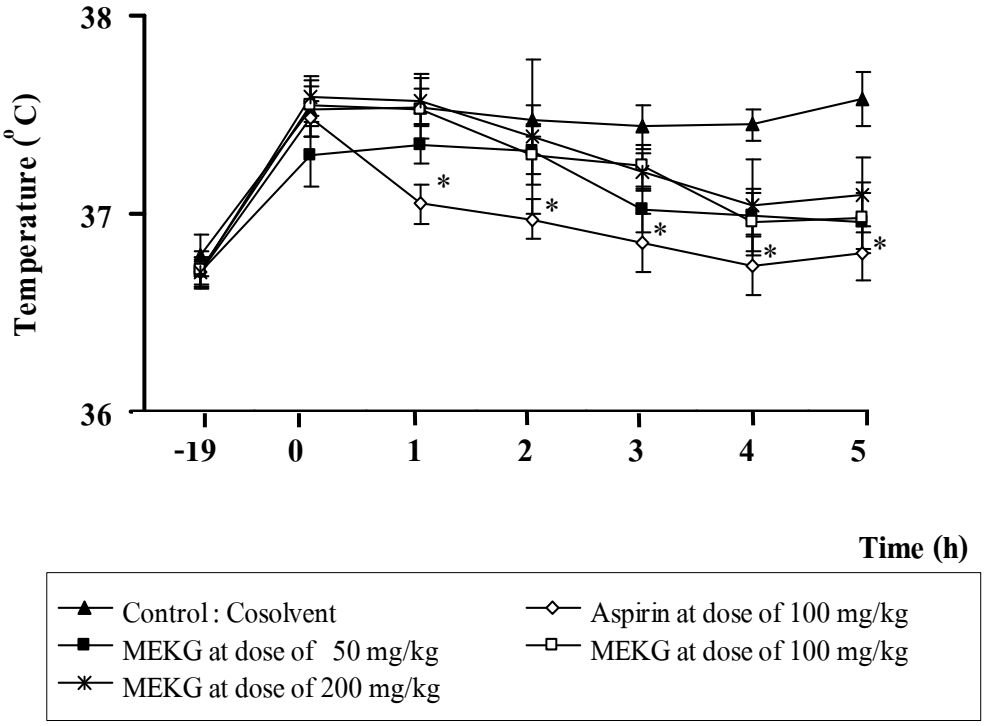

Figure 26. Effects of the methanol extract of *Kaempferia galanga* (MEKG) and aspirin on the brewer's yeast induced pyrexia in rats.

$^{*}p<0.05$, significantly different compared to the control (Bonferroni's test).

3. Anti-inflammatory activity

3.1 Carrageenan-induced rat paw edema

In the carrageenan-induced rat paw edema test, the average right hind paw volumes and percentages of inhibition by the extracts and standard drugs are showed in Table 14 and Figure 27. For the control group, the injection of the phlogistic agent caused localized edema starting at 0.5 h after injection. The swelling increased progressively to a maximum volume of 6.62 ± 0.11 ml at 3 h after the carrageenan injection. Rats pre-treated with aspirin (100 mg/kg, p.o.) had a significant reduction of the paw edema at 2 h (up to 5 h) after the administration and the percentage of inhibition being 25, 25.4, 24 and 24.3 respectively compared to the control. Only the extract at doses of 100 and 200 mg/kg exhibited the anti-inflammatory effect to reduce the paw edema volumes. *K. galanga* at the oral dose of 100 mg/kg significantly reduced paw edema volume at 3 ($p<0.05$), and 4 ($p<0.01$) h with percentage of inhibition being 14.2 and 13, respectively when compared to the control. *K. galanga* at dose of 200 mg/kg was more potent than *K. galanga* at dose of 100 mg/kg, which significantly decreased paw edema volume at 2 ($p<0.05$), 3 ($p<0.01$), 4 ($p<0.01$) and 5 ($p<0.01$) h with percentage of inhibition being 13.4, 16.5, 16.4 and 17.0, respectively compared to the control. Therefore, the results indicated that *K. galanga* (100 and 200 mg/kg) possessed anti-inflammatory activity and had maximum inhibitory effects at 3 h similar to the standard aspirin.

Table 14. Effect of the methanol extract of *Kaempferia galanga* (MEKG) and aspirin on carrageenan-induced paw edema in rats.

Treatment	Dose	Initial paw	Paw edema volume (ml)					Inhibition of paw edema (%)			
	(mg/kg, p.o.)	volume (ml)	1 h	2 h	3 h	4 h	5 h	2 h	3 h	4 h	5 h
Cosolvent		3.91±0.22	5.38±0.25	6.31±0.23	6.62±0.11	6.56±0.10	6.55±0.10				
Aspirin	100	3.87±0.17	4.60±0.14	4.73±0.16**	4.94±0.18**	4.99±0.14**	4.95±0.14**	25.04	25.41	23.96	24.31
MEKG	50	3.88±0.19	5.15±0.24	5.59±0.16	5.75±0.28	5.91±0.21	6.03±0.12	11.45	13.25	9.82	7.89
MEKG	100	3.87±0.17	4.96±0.18	5.51±0.21	5.68±0.23*	5.71±0.15**	5.93±0.18	12.74	14.23	13.00	9.40
MEKG	200	4.02±0.17	5.13±0.18	5.47±0.21*	5.53±0.21**	5.48±0.24**	5.44±0.20**	13.39	16.46	6.39	16.96

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

* $p<0.05$, ** $p< 0.01$, significantly different compared to the control (Bonferroni's test).

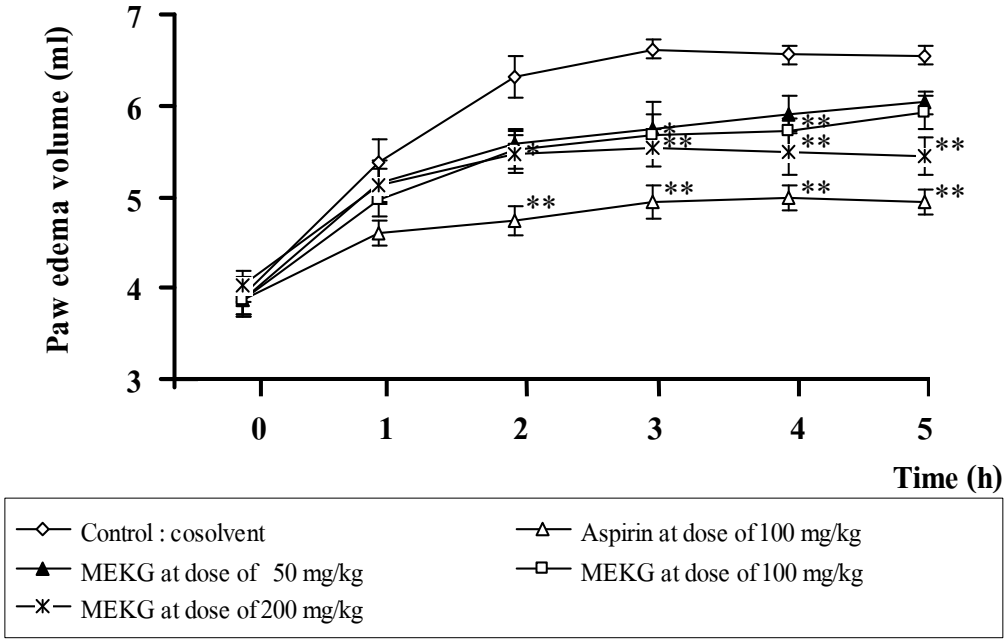

Figure 27. Effects of the methanol extract of *Kaempferia galanga* (MEKG) and aspirin on carrageenan-induced paw edema in rats.

$^{*}p<0.05$, $^{**}p< 0.01$, significantly different compared with control (Bonferroni's test)

3.1 Cotton pellet-induced granuloma formation

K. galanga extract at doses of 50, 100 and 200 mg/kg exhibited a significant and dose-related inhibition of transudative weight (52.38±0.67, 46.50±0.43 and 42.15±0.40; $p<0.01$, respectively) and granuloma weight (1.47±0.01, 1.37±0.02 and 1.25±0.01; $p<0.01$, respectively) with the percentage of inhibition being 12, 18 and 25, respectively compared to the control. However, the aspirin (100 mg/kg, p.o.) exhibited a significantly reduced both in transudative weight (38.13±1.06, $p<0.01$) and granuloma weight (1.22±0.01, $p<0.01$) when compared to the control.

Table15. Effects of the methanol extract of *Kaempferia galanga* (MEKG) and aspirin on cotton pellet-induced granuloma formation in rats.

Treatment	Dose (mg/kg, p.o.)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)	GI (%)
Control		94.18±0.68	33.53±0.38	60.65±0.58	0.68±0.02	-
Aspirin	100	62.61±1.16*	24.49±0.22*	38.13±1.06*	0.22±0.01*	27
MEKG	50	81.81±0.70*	29.44±0.25*	52.38±0.67*	0.47±0.01*	12
MEKG	100	73.53±0.51*	27.38±0.36*	46.50±0.43*	0.37±0.02*	18
MEKG	200	67.16±1.56*	25.01±0.15*	42.15±0.40*	0.25±0.01*	25

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

*p< 0.01, significantly different compared with control group (Bonferroni's test).

GI : Granuloma inhibition

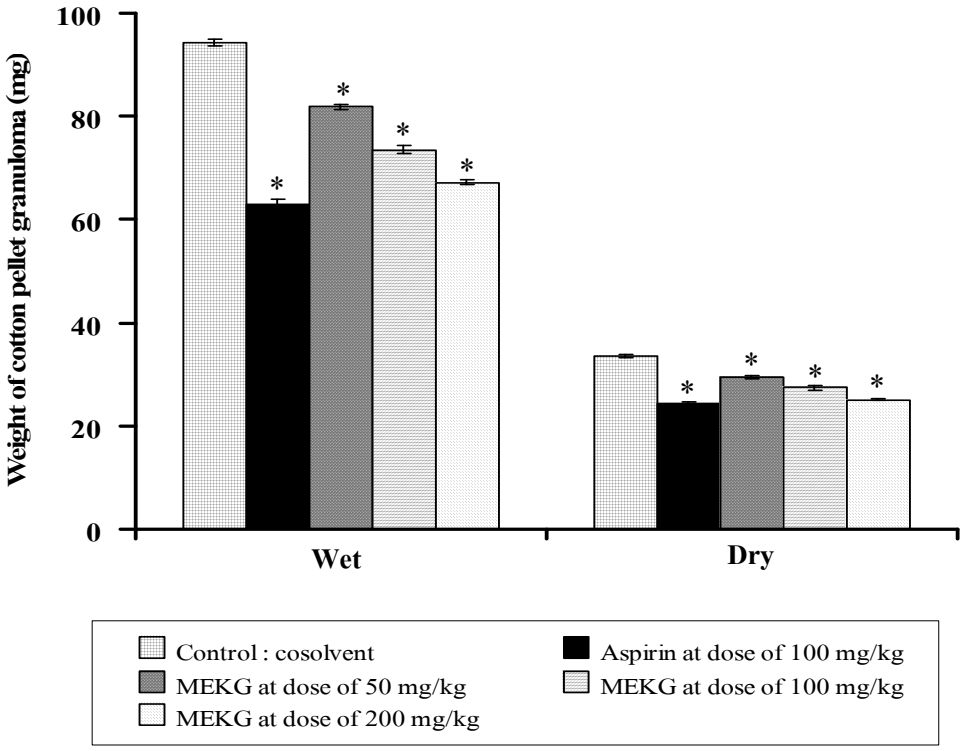

Figure 28. Effects of the methanol extract of *Kaempferia galanga* (MEKG) and aspirin on cotton pellet-induced granuloma formation in rats.

* $p < 0.01$, significantly different compared to the control (Bonferroni's test).