CHAPTER 4

RESULTS

1. Acute toxicity

The methanol extract of *Piper sarmentosum* leaves (MEPS) which orally administered at the dose of 5 g/kg to mice (10 males and 10 females) did not change in the behavioral responses during the observation period. No mortality was observed up to 7 days of monitoring.

2. Analgesic activity

2.1 Hot plate test

The results showed that MEPS at doses of 50 and 100 mg/kg did not significantly alter the reaction time at 30, 45, 60, 75 and 90 minutes after administration when compared with control, whereas MEPS at dose of 200 mg/kg slightly significantly delayed the response of animal to hot plate thermal stimulation at 60, 75 and 90 minutes ($p<0.05$) after administration compared with control. Morphine at the dose of 5 mg/kg, a reference analgesic drug, significantly reduced the reaction time to hot plate test at all time intervals measured (30, 45, 60, 75 and 90 minutes) after administration ($p<0.01$). So, the results indicated that MEPS only at dose of 200 mg/kg decreased the latency of nociceptive response for the thermal stimulation by hot plate test in mice (Table 5, Figure 19).

The antagonistic action of naloxone on effects of morphine or MEPS on the latentcy of nociceptive response in hot plate test was also studied. The results showed that naloxone at dose of 2 mg/kg, i.p. given before morphine (5 mg/kg, s.c.) completely antagonized the effect of morphine at all time intervals measured ($p<0.01$). Furthermore, naloxone (2 mg/kg, i.p.) given before MEPS at dose of 200 mg/kg significantly delayed the latency of nociceptive response of the extract in hot plate test at 60 ($p<0.05$) and 75 minutes ($p<0.01$) when compared with MEPS at dose of 200 mg/kg alone (Table 6, Figure 20).

Table 5. Effects of the methanol extract of *Piper sarmentosum* leaves (MEPS) and morphine on the latency of nociceptive response-induced by heat in mice.

Treatment	Dose (mg/kg)	Latency of nociceptive response (s)				
	p.o.	30 min	45 min	60 min	75 min	90 min
Control (Distilled water)		8.57±0.33	8.72±0.22	8.70±0.31	9.14±0.24	8.47±0.31
Morphine sulfate	5, s.c.	13.07±0.72**	18.68±0.76**	23.30±0.56**	26.32±0.50**	20.25±0.24**
MEPS	50	8.25±0.22	8.70±0.16	8.74±0.28	9.08±0.21	8.60±0.31
MEPS	100	8.33±0.38	8.62±0.24	8.74±0.13	8.86±0.40	8.89±0.30
MEPS	200	8.28±0.27	8.74±0.25	10.23±0.27*	10.68±0.33*	9.88±0.37*

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

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

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

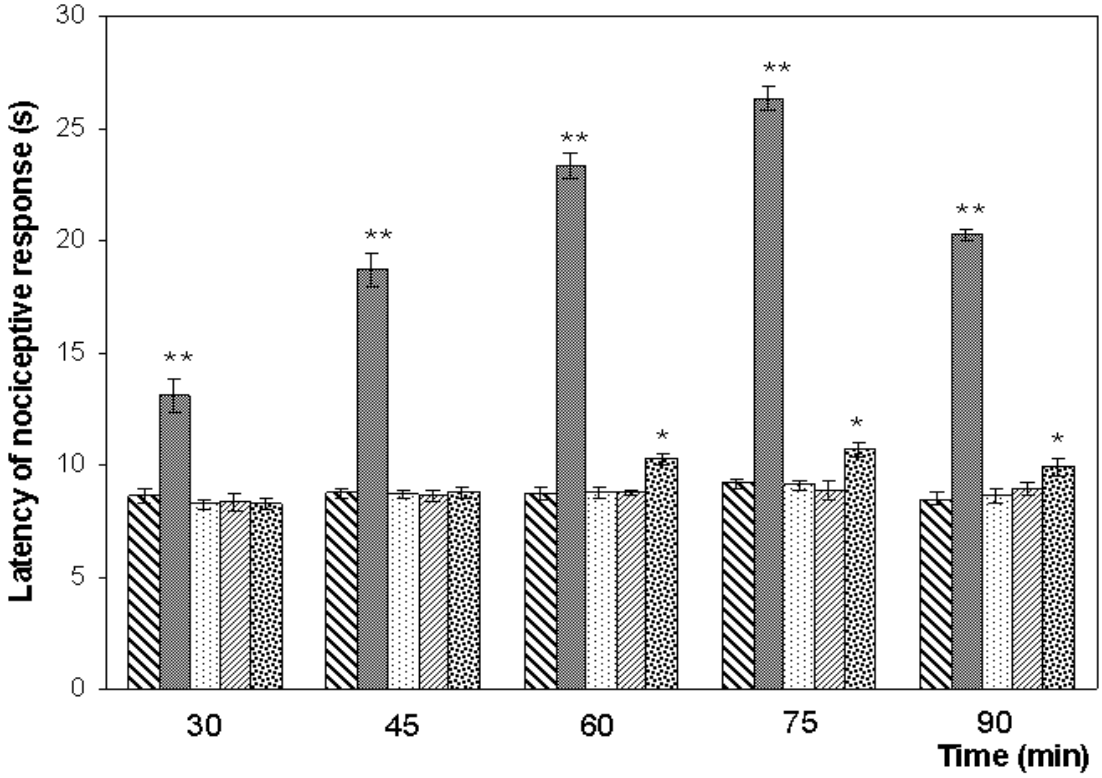

Figure 19. Effects of the methanol extract of *Piper srmentosum* leaves (MEPS) (50, 100 and 200 mg/kg) and morphine, s.c. on the latency of nociceptive response-induced by heat in mice.

$^{*}p$ <0.05, $^{**}p$ <0.01, significantly different compared with control (Bonferroni's test). Control: distilled water (▨), Morphine at dose of 5 mg/kg (▩), MEPS at dose of 50 mg/kg (⁞⁞), MEPS at dose of 100 mg/kg (▨), MEPS at dose of 200 mg/kg (▩)

Table 6. Antagonistic effects of naloxone with morphine and methanol extract of *Piper sarmentosum* leaves (MEPS) at dose of 200 mg/kg on the latency of nociceptive response-induced by heat in mice.

Treatment	Dose (mg/kg)	Latency of nociceptive response (s)				
		30 min	45 min	60 min	75 min	90 min
Morphine	5, s.c.	13.07±0.72	18.68±0.76	23.30±0.56	26.32±0.50	20.25±0.24
Naloxone	2, i.p.					
+ Morphine	5, s.c.	9.31±0.53[a]	9.83±0.60[a]	9.74±0.64[a]	9.28±0.50[a]	8.92±0.40[a]
MEPS	200, p.o.	8.28±0.27	8.74±0.25	10.23±0.27	10.68±0.33	9.88±0.36
Naloxone	2, i.p.					
+ MEPS	200, p.o.	8.99±0.31	9.34±0.56	9.15±0.36[#]	8.97±0.34[##]	9.28±0.37

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

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

Latency of nociceptive response was initially measured 10 min after intraperitoneal administration of naloxone.

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

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

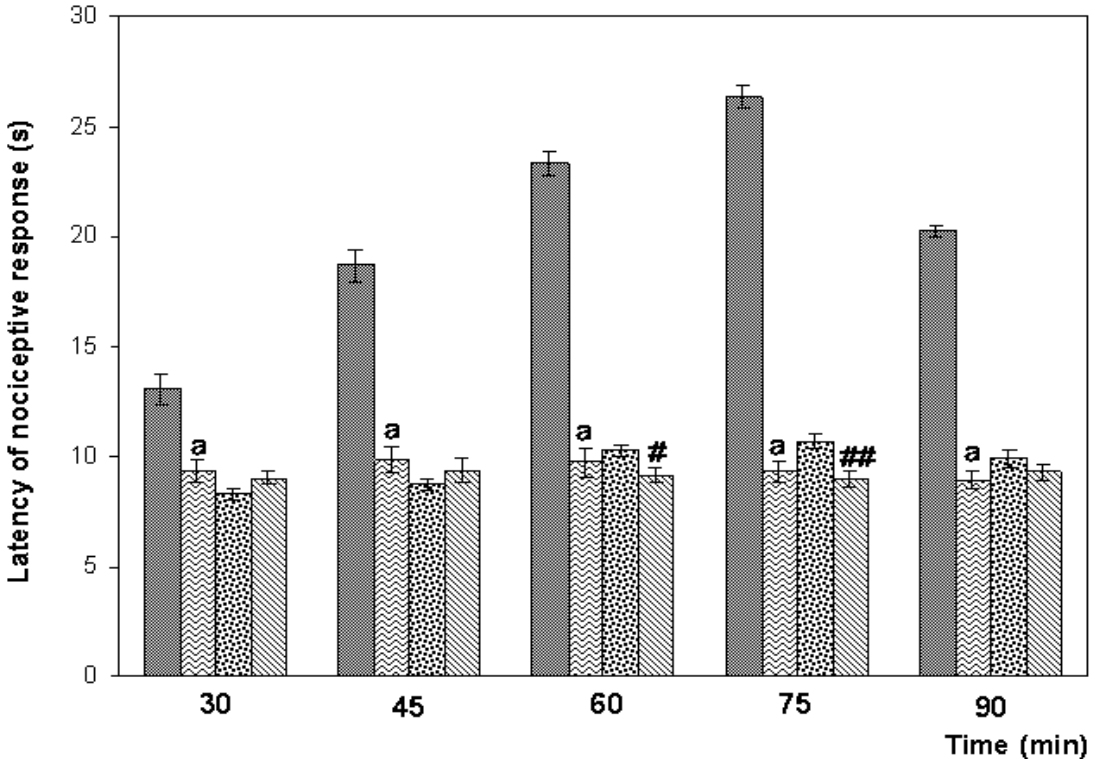

Figure 20. Antagonistic effects of naloxone (2 mg/kg, i.p.) with morphine (5 mg/kg, s.c.) and methanol extract of *Piper srmentosum* leaves (MEPS) at dose of 200 mg/kg, orally on the latency of nociceptive response-induced by heat in mice.

^{a}p <0.01, significantly different compared with morphine (Independent *t*-test).

$^{\#}p$ <0.05, $^{\#\#}p$ <0.01, significantly different compared with MEPS at dose of 200 mg/kg (Independent *t*-test).

Morphine at dose of 5 mg/kg (▓), Naloxone at dose of 2 mg/kg + Morphine at dose of 5 mg/kg (▒), MEPS at dose of 200 mg/kg (▒), Naloxone at dose of 2 mg/kg + MEPS at dose of 200 mg/kg (▒)

2.2. Tail flick test

In the present study, the results revealed that only the MEPS at dose of 200 mg/kg, orally significantly produced an antinociceptive effect at the time 45 (p<0.05), 60 (p <0.01), 75 (p<0.05) and 90 (p<0.05) minutes after administration compared with control. Morphine (5 mg/kg, s.c.), a reference analgesic drug, significantly delayed the reaction time at all time interval measured in tail flick test in rats. All results are shown in Table 7, Figure 21.

In antagonistic studies of naloxone, the result 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 time intervals measured (p<0.01). In addition, naloxone at dose of 2 mg/kg, i.p. given before MEPS at dose of 200 mg/kg significantly delayed the latency of nociceptive response produced by the extract in tail flick test at 45 (p<0.05), 60 (p<0.05), 75 (p<0.01) and 90 (p<0.01) minutes when compared with MEPS at dose of 200 mg/kg (Table 8, Figure 22).

Table 7. Effects of the methanol extract of *Piper sarmentosum* leaves (MEPS) and morphine on the latency of nociceptive response-induced by light beam in rats.

Treatment	Dose (mg/kg)	Latency of nociceptive response (s)				
	p.o.	30 min	45 min	60 min	75 min	90 min
Control (Distilled water)		1.63±0.04	1.69±0.04	1.69±0.03	1.72±0.06	1.71±0.06
Morphine sulfate	5, s.c.	2.50±0.07**	4.24±0.07**	4.46±0.07**	3.60±0.07**	3.23±0.08**
MEPS	50	1.73±0.03	1.69±0.05	1.65±0.03	1.80±0.07	1.76±0.05
MEPS	100	1.75±0.06	1.73±0.04	1.78±0.04	1.76±0.07	1.79±0.06
MEPS	200	1.83±0.05	1.94±0.07*	1.95±0.06**	2.04±0.07*	2.02±0.06

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

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

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

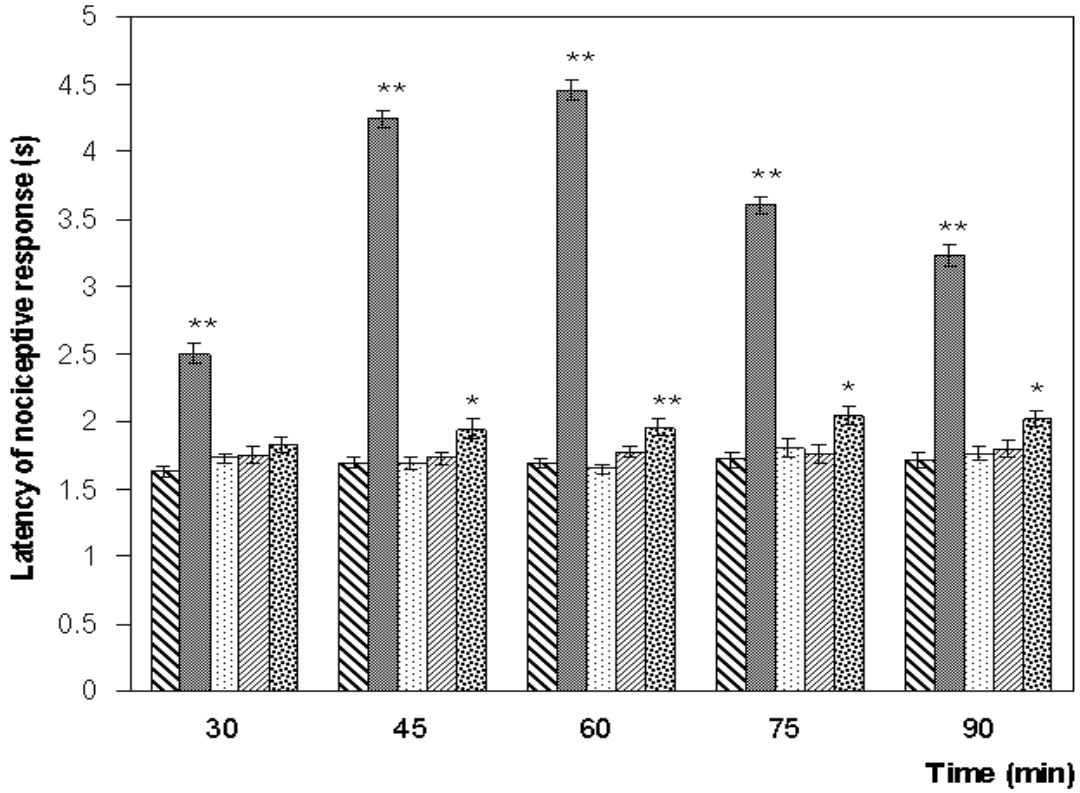

Figure 21. Effects of the methanol extract of *Piper srmentosum* leaves (MEPS) (50, 100 and 200 mg/kg) and morphine, s.c. on the latency of nociceptive response-induced by light beam in rats.

$^{*}p$ <0.05, $^{**}p$ < 0.01, significantly different compared with control (Bonferroni's test). Control: distilled water (▨), Morphine at dose of 5 mg/kg (▩), MEPS at dose of 50 mg/kg (▧), MEPS at dose of 100 mg/kg (▨), MEPS at dose of 200 mg/kg (▩)

Table 8. Antagonistic effects of naloxone with morphine and methanol extract of *Piper sarmentosum* leaves (MEPS) at dose of 200 mg/kg on the latency of nociceptive response-induced by light beam in rats.

Treatment	Dose (mg/kg)	Latency of nociceptive response (s)				
		30 min	45 min	60 min	75 min	90 min
Morphine	5, s.c.	2.50±0.07**	4.24±0.07**	4.46±0.07**	3.60±0.07**	3.23±0.08**
Naloxone	2, i.p.					
+ Morphine	5, s.c.	1.65±0.03a	1.61±0.03a	1.71±0.04a	1.68±0.04a	1.63±0.05a
MEPS	200	1.83±0.05	1.94±0.07	1.95±0.06	2.04±0.07	2.02±0.06
Naloxone	2, i.p.					
+ MEPS	200	1.77±0.08	1.71±0.05#	1.73±0.05#	1.76±0.04##	1.78±0.05##

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

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

Latency of nociceptive response was initially measured 10 min after intraperitoneal administration of naloxone

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

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

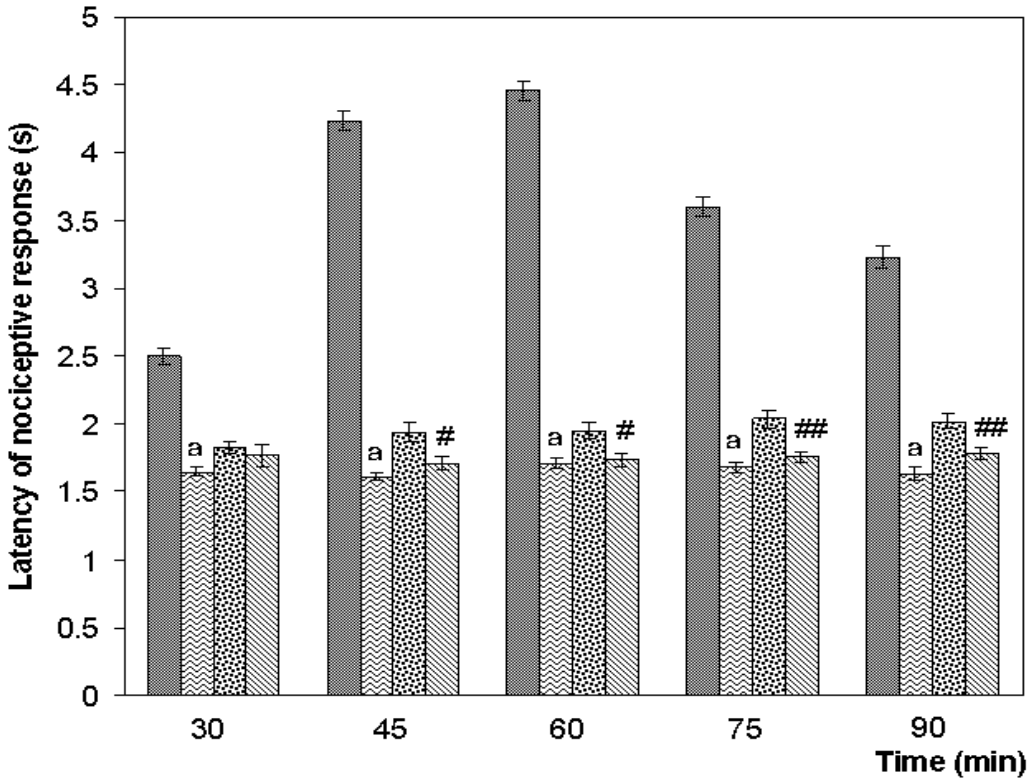

Figure 22. Antagonistic effects of naloxone (2 mg/kg, i.p.) with morphine (5 mg/kg, s.c.) and methanol extract of *Piper srmentosum* leaves (MEPS) at dose of 200 mg/kg, orally on the latency of nociceptive response-induced by light beam in rats.

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

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

Morphine at dose of 5 mg/kg (▩), Naloxone at dose of 2 mg/kg + Morphine at dose of 5 mg/kg (▨), MEPS at dose of 200 mg/kg (▩), Naloxone at dose of 2 mg/kg + MEPS at dose of 200 mg/kg (▨)

2.3. Writhing test

The methanol extract of *Piper sarmentosum* from leaves (MEPS) at doses of 50, 100 and 200 mg/kg significantly inhibited the number of writhes induced by 0.6% acetic acid compared with control. The number of writhing produced by a standard drug aspirin at dose of 200 mg/kg significantly decreased the number of writhes produced by 0.6% acetic acid compared with control, and the percentage of inhibition was 61.37% (20.9±1.9 vs 54.1±1.2, $p<0.01$). MEPS at doses of 50, 100 and 200 mg/kg significantly decreased the number of writhing produced by 0.6% acetic acid (47.4±1.5 vs 54.1±1.2, $p<0.05$; 41.1±1.6 vs 54.1±1.2, $p<0.01$; 37.20 ±1.6 vs 54.1±1.2, $p<0.01$, respectively) with percentage of inhibition by 12.38, 24.02 and 31.24, respectively when compared with control. The resulted showed that the potency of the MEPS at dose of 200 mg/kg to reduce the number of writhing was more than those of MEPS at doses of 50 and 100 mg/kg. The activity of MEPS to reduce the number of writhing was likely to be dose-related (Table 9 and Figure 23).

Table 9. Effects of the methanol extract of *Piper sarmentosum* leaves (MEPS) and aspirin on the number of writhings induced by 0.6% acetic acid in mice.

Treatment	Dose (mg/kg), p.o.	Number of writhings	Inhibition (%)
Control (DW 10 ml/kg)		54.1±1.2	0
Aspirin	200	20.9±1.9**	61.37
MEPS	50	47.4±1.5*	12.38
MEPS	100	41.1±1.6**	24.02
MEPS	200	37.2±1.6**	31.24

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

Thirty minutes after treatment, mice were intraperitoneally injected with 0.6% (v/v) acetic acid. Number of writhings measured in a 20-minute period after intraperitonelly injection of 0.6% (v/v) acetic acid

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

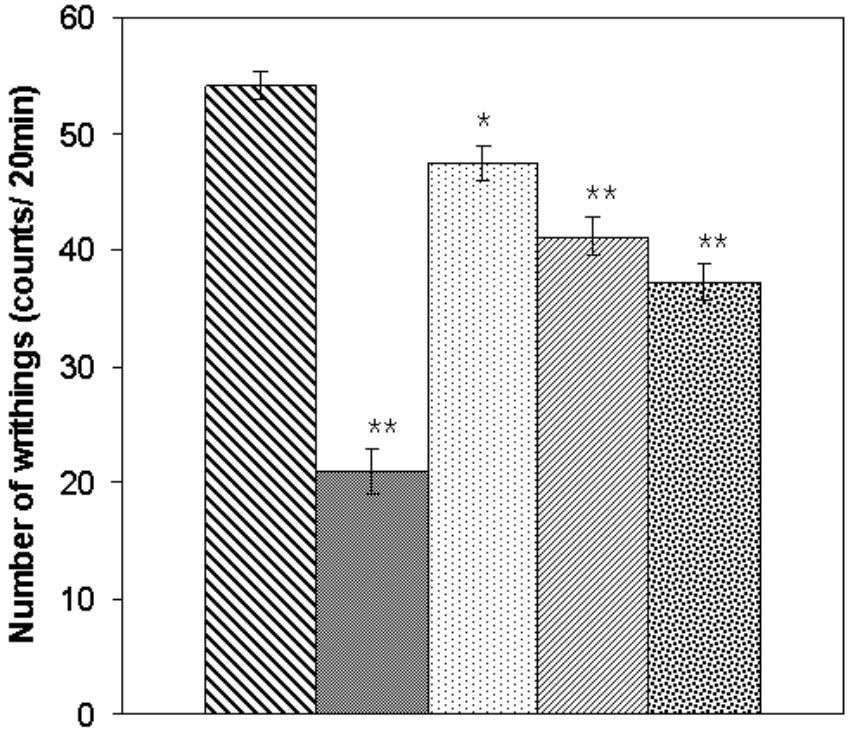

Figure 23. Effects of the methanol extract of *Piper sarmentosum* leaves (MEPS) and aspirin on the number of writhings induced by 0.6% acetic acid in mice.
$^{*}p$ <0.05, $^{**}p$ <0.01, significantly different compared with control (Bonferroni's test).
Control: distilled water (▨), Aspirin at dose of 200 mg/kg (▩), MEPS at dose of 50 mg/kg (⁞⁞), MEPS at dose of 100 mg/kg (▨), MEPS at dose of 200 mg/kg (▩).

2.4. Formalin test

After the standard drug morphine (5 mg/kg) and aspirin (200 mg/kg) given before injection of 20 µl of 2.5% formalin for 30 minutes, the licking time (sec) in early phase (0-5 min) significantly decreased by 65.54% (77.34±1.52 vs 26.65±1.87; $p<0.01$) and 28.47% (77.34±1.52 vs 55.32±2.17; $p<0.05$) when compared with control, whereas only MEPS at dose of 200 mg/kg significantly decreased the licking time (s) in early phase (0-5 min) by 10.96% (77.34±1.52 vs 68.86±1.84 $p<0.05$) when compared with control. In late phase (15-30 min), morphine at dose of 5 mg/kg and aspirin at dose of 200 mg/kg significantly reduced the licking time (s) induced by 2.5% formalin by 80.86% (100.81±2.63 vs 19.30±1.71; $p<0.01$) and 52.9% (100.81±2.63 vs 47.48±3.34; $p<0.01$), respectively when compared with control, whereas only MEPS at doses of 100 and 200 mg/kg significantly decreased the licking time (s) by 12.60% (100.81±2.63 vs 88.11± 3.18; $p<0.05$) and 21.96 % (100.81±2.63 vs 78.67±3.00; $p<0.01$), respectively when compared with control. The results indicated only MEPS at dose of 100 mg/kg significantly reduced the licking time in late phase (15-30 min) when compared with control, whereas MEPS at dose of 200 mg/kg significantly reduced the licking time both in early and late phase (Table 10, Figure 24).

Table 10. Effects of the methanol extract of *Piper sarmentosum* leaves (MEPS) and aspirin on the

nociceptive response by formalin-induced paw licking in mice.

Treatment	Dose (mg/kg) p.o.	Licking of the hindpaw (s)			
		Early phase (0-5 min)	Inhibition (%)	Late phase (15-30 min)	Inhibition (%)
Control (DW 10 ml/kg)		77.34±1.52	0	100.81±2.63	0
Morphine	5, s.c.	26.65±1.87**	65.54	19.30±1.71**	80.86
Aspirin	200	55.32±2.17**	28.47	47.48±3.34**	52.90
MEPS	50	78.21±2.10	-1.12	98.01±2.43	2.78
MEPS	100	75.92±2.03	1.84	88.11±3.18*	12.60
MEPS	200	68.86±1.84*	10.96	78.67±3.00**	21.96

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

Thirty minutes after treatment, mice were subcutaneously injected to hindpaw with 2.5% (v/v) formalin

Fifteen minutes after morphine, mice were subcutaneously injected to hindpaw with 2.5% (v/v) formalin

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

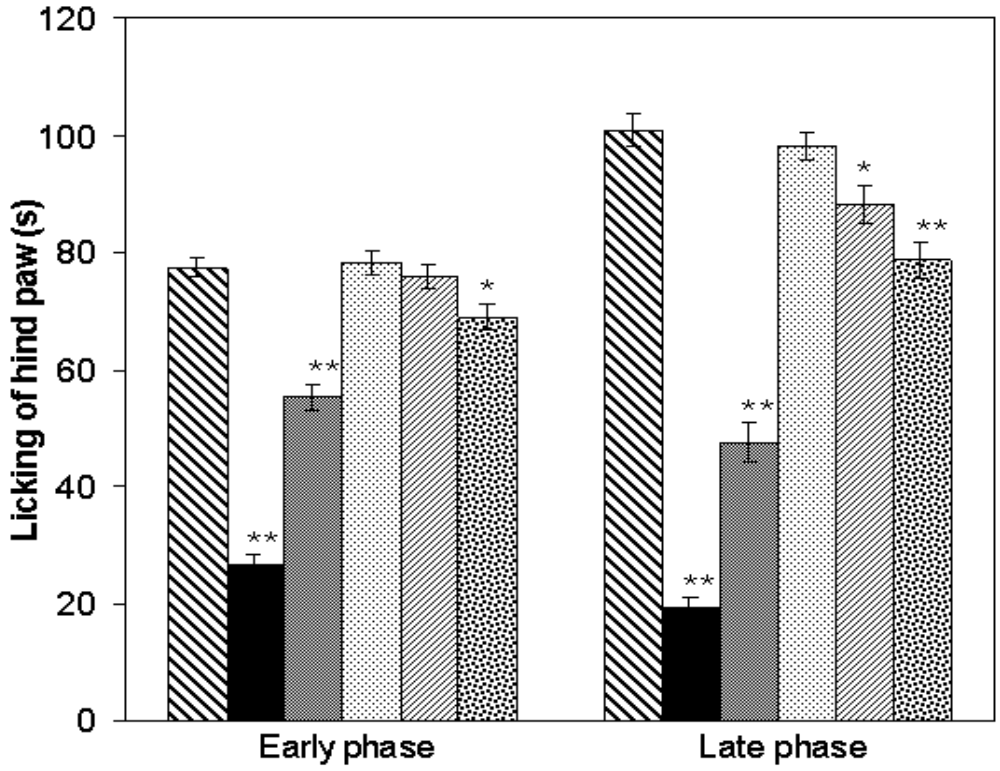

Figure 24. Effects of the methanol extract of *Piper sarmentosum* leaves (MEPS) and aspirin on nociceptive response by formalin-induced paw licking in mice.

$^{*}p$ <0.05, $^{**}p$ <0.01, significantly different compared with control (Bonferroni's test). Control: distilled water (▨), Morphine at dose of 5 mg/kg(■), Aspirin at dose of 200 mg/kg (▩), MEPS at dose of 50 mg/kg (⬚), MEPS at dose of 100 mg/kg (▨), MEPS at dose of 200 mg/kg (▨).

3. Anti-inflammatory activity

The standard drug aspirin at the dose of 200 mg/kg significantly decreased the volume of hind paw edema at 1, 2, 3, 4 and 5 h (p<0.01) with percentage of inhibition by 15.6, 31.8, 33.3, 30.4 and 30.2, respectively when compared with control. The MEPS at the dose of 50 mg/kg significantly reduced the volume of paw edema only at 3 h (p<0.05) with percentage of inhibition by 8.6, and at the dose of 100 mg/kg significantly reduced the volume of paw edema at 3 (p<0.01) and 4 h (p<0.05) with percentage of inhibition by 18.6 and 9.5, respectively whereas the MEPS at 200 mg/kg significantly decreased the volume of paw edema at 2 , 3, 4 and 5 h (p <0.01) with percentage of inhibition by 11.8, 24.7, 14.1 and 11.9, respectively after administration when compared with control. Therefore, the results indicated that MEPS (50-200 mg/kg) used in this study possessed anti-inflammatory activity. All results were presented in Table 11 and Figure 25.