

# รายงานการวิจัย เรื่อง

## Studies on Analgesic, Antipyretic and Anti-inflammatory Activities of Methanol Extract of *Piper sarmentosum* Leaves in Experimental Animals

โดย

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# **Studies on analgesic, antipyretic and anti-inflammatory activities of methanol extract of *Piper sarmentosum* leaves in experimental animals**

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## **ABSTRACT**

The methanolic extract of *Piper sarmentosum* Roxb. leaves at doses of 50, 100 and 200 mg/kg was investigated for its analgesic, antipyretic and anti-inflammatory activities in mice and rats. Only the dose of 200 mg/kg p.o. of the extract showed a significant analgesic activity both in hot plate and tail flick tests. Naloxone 2 mg/kg i.p. decreased the latency of nociceptive response produced by the extract. The extract at doses of 50, 100, and 200 mg/kg p.o. significantly reduced the number of writhings and stretchings induced by acetic acid. Only the dose of 200 mg/kg p.o. of the extract significantly decreased the licking activity both in early and late phase in formalin test. The overall results indicated that the extract possesses analgesic activity which the mechanisms were probably due to centrally and peripherally-mediated on central nervous system pathways. In carrageenan-induced rat paw edema test, the extract showed a significant anti-inflammatory effect at doses of 50, 100 and 200 mg/kg p.o. No antipyretic effect of the extract on yeast-induced pyrexia was observed. In acute toxicity test, the LD<sub>50</sub> value observed was more than 5 g/kg p.o.

*Keywords:* *Piper sarmentosum*; Methanol extract; Analgesic; Antipyretic; Anti-inflammatory

## 1. Introduction

*Piper sarmentosum* Roxb., a Piperaceae, commonly known as “Cha Plu” in local Thai name, is a plant widely distributed throughout every region in Thailand [1]. The plant is a terrestrial herb with 60 cm high, green trunk and jointed at the nodes. The leaves are thin, dark green, heart shaped and spicy tasted. The previous pharmacological studies of *Piper sarmentosum* extract showed that it possesses an antimicrobial activity against *Escherichia coli* and *Bacillus subtilis* [2], a hypoglycemic effect [3], a neuromuscular blocking activity in rat phrenic nerve-hemidiaphragm preparation [4], an antiplasmodial activity against *Plasmodium falciparum* and *Plasmodium berghei* [5] and an antiprotozoal effect against *Entamoeba histolytica* [6].

The previous published data have been reported that various parts of this plant contain many biologically active compounds such as asaricin,  $\alpha$ -asarone [2], hydrocinnamic acid,  $\beta$ -sitosterol [7], sarmentine, sarmentosine [8], vitamin C, E and carotenes [9], longifolene,  $\beta$ -caryophyllene, allo-aromadendrene, 9-epi-(E)-caryophyllene,  $\beta$ -asarone, viriflorene and  $\beta$ -selinene [10], guineensine, brachystamide B, brachyamide B, sesamin, methyl piperate and 1-piperrettyl pyrrolidine [11]. In folk medicine, the plant was applied to remedy for toothache, headache, asthma, rheumatism, and to reduce fever in influenza patients [10,11]. From the pharmacodynamics point of view, the present work was undertaken to investigate analgesic, antipyretic and anti-inflammatory activities of the methanol extract of *P. sarmentosum* leaves to verify the traditional use in herbal medicine and explain possible mechanisms of the methanolic extract of *P. sarmentosum* leaves in comparison with reference drugs, morphine and aspirin.

## 2. Experimental

### 2.1. Plant material

The fresh leaves of *P. sarmentosum* Roxb. were collected in March, 2005 from Ranod district, Songkla Province, Thailand. The taxonomical identification of this plant was identified by Assist. Prof. Chuotip Purintaworakul, Botany section, Department of Biology, Prince of Songkla University, Thailand. A voucher specimen was preserved in our laboratory for future reference.

### 2.2. Preparation of the extract and standard drugs

The 40 kg fresh leaves of *P. sarmentosum* were cleaned with tap and distilled water, respectively and air-dried at room temperature. The dried leaves were pulverized by an electric blender to give 6 kg of a fine powder. Then, all powder was extracted using cold extraction by macerating in 20 L of methanol and allowed to stand for 7 days at room temperature. This extraction process was repeated 2 times. All of the extract collected was filtered, and evaporated under reduced pressure condition to give 480 ml of the black viscous and oil-like extract mixture. This extract mixture was lyophilized by freeze-dried to give a total solid residue of 270 g (yield 0.675%w/w) which was stored in a closed bottle and kept in a refrigerator at temperature below 4 °C. The methanol extract of *P. sarmentosum* leaves at doses of 50, 100 and 200 mg/kg was prepared by dissolving in 0.9% NSS. The standard drugs used in this study were aspirin (200 mg/kg p.o.), morphine sulphate (5 mg/kg s.c.) and naloxone (2 mg/kg i.p.). All standard drugs were prepared by dissolving in 0.9% NSS.

### 2.3. *Experimental animals*

Male Swiss albino mice (28-40 g) and Wistar rats (140-170 g) were used for the experiments. The animals were obtained from the Southern Laboratory Animal Facility, Prince of Songkla University, Hat-Yai, Songkhla, Thailand, and kept in the room which was maintained environmental conditions of 23-26 °C and 12 h-light/dark cycle. The animals were fed on a standard rodent diet with water *ad libitum*.

### 2.4. *Acute toxicity*

The 50% lethal dose (LD<sub>50</sub>) of the methanol extract of *P. sarmentosum* leaves was estimated by up-and-down method in mice [12]. The methanol extract of *P. sarmentosum* leaves at dose of 5 g/kg p.o. was administered to each group of male and female mice (each sex consists of 10 mice). Behavioral parameters such as convulsion, hyperactivity, sedation, grooming, loss of righting reflex and increased or decreased respiration were closely observed during a period of 8 hours and 7 days after administration. Food and water were given *ad libitum*.

### 2.5. *Analgesic activity*

#### 2.5.1. *Hot plate test*

The hot plate test was carried out according to the method described by Woolfe and MacDonald [13]. Mice were randomly assigned to eight groups of ten animals each. The first group (control) received distilled water 10 ml/kg p.o. Morphine (5 mg/kg s.c.), naloxone (2 mg/kg i.p.) were given to the second and third group which served as standard. The methanol extract of *P. sarmentosum* leaves was given at doses of 50, 100 and 200 mg/kg p.o. to the fourth, fifth and sixth group, respectively. The antagonistic of naloxone (2 mg/kg i.p.) given 10 min before morphine (5 mg/kg s.c.) or the extract at dose of 200 mg/kg p.o. was assigned to the seventh and eighth group, respectively.

After 30 min of treatment with each extract dose (15 min for morphine and 10 min for naloxone), mice were placed on a hot plate maintained the temperature at  $55^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . Latency of nociceptive response such as licking, flicking of a hind limb or jumping was measured. The nociceptive response was measured at 30, 45, 60, 75 and 90 min. In antagonistic study, naloxone, an antagonist of morphine, given 10 min before a standard drug morphine (5 mg/kg s.c.) and the extract at dose of 200 mg/kg p.o. was assigned to the seventh and eight group, respectively.

### 2.5.2. Tail flick response

The tail flick test used in this experiment was described by D'Amour and Smith [14]. Briefly, rats were randomly assigned to eight groups of ten animals each. The experiment was carried out in the same way as the hot plate test including of all drugs used for the experiments. After 30 min of treatment with each extract dose (15 min for morphine and 10 min for naloxone), rats were gently manipulated and placed on the tail flick apparatus by keeping their tails smoothed into the light on the distal one-third position of the animal tail. Latency of nociceptive response was recorded when the rat flicked its tail. The nociceptive response was measured every 15 min over a 60-min period.

### 2.5.3. Acetic acid-induced writhing response

The activity of the methanol extract of *P. sarmentosum* leaves on acetic acid-induced writhing response was investigated according to Koster et al. [15]. Mice were randomly assigned to five groups of ten animals each. The first group (control) received distilled water 10 ml/kg p.o. Aspirin at dose of 200 mg/kg p.o. was given to the second group as a standard drug. The plant extract at doses of 50, 100 and 200 mg/kg p.o. were

given to the third, fourth and fifth group. After 30 min of treatment, 0.6% (v/v) acetic acid at the dose of 10 ml/kg was injected intraperitoneally. The number of writhings and stretchings was counted over a 20-min period. A reduction in the writhing number compared to control group was evaluated for the analgesia which was expressed as % inhibition of writhings.

#### 2.5.4. Formalin test

The method of Hunskaar et al. [16] was used. Mice were randomly divided into six groups of ten animals each. The distilled water (10 ml/kg p.o.) was given to the first group (control). Morphine sulphate (5 mg/kg s.c.), aspirin (200 mg/kg p.o.) and the plant extract at doses of 50, 100, and 200 mg/kg were given to the second, third, fourth, and sixth group, respectively. After 30 min of treatment, a 20  $\mu$ l of 2.5% formalin was injected subcutaneously to hindpaw. The licking time in early phase (0-5 min) and late phase (15-30 min) were recorded after formalin injection.

#### 2.6. Anti-inflammatory activity

##### *Carrageenan-induced rat paw edema*

The anti-inflammatory activity was studied according to the method described by Winter et al. [17]. The rats were divided into five groups of ten animals each. The initial right hind paw volume of rats was measured and recorded using a Plethysmometer. Edema was induced by subplantar injection of 0.1 ml of 1% (w/v) freshly prepared of carrageenan in 0.9% NSS into the right hind paw of each rat. The distilled water (10 ml/kg p.o) was given to the first group (control). Aspirin (200 mg/kg p.o. as a standard drug) and the plant extract at doses of 50, 100 and 200 mg/kg p.o. were given to the second, third, fourth and fifth group, respectively. After 30 min of treatment, a 0.1 ml of

1%(w/v) carrageenan in 0.9% NSS was subcutaneously injected into the subplantar region of the rat hindpaw. The volume of hindpaw was measured using at time 0.5, 1, 2, 3, 4 and 5 hours after carrageenan injection.

### 2.7. Antipyretic activity

Antipyretic activity was studied using the method described by Adam et al. [18] with a minor modification. The experiments were performed in the same way as carrageenan-induced rat paw edema. Shortly, all rats used in the experiment were fasted overnight with water *ad libitum* before the beginning of experiment. Pyrexia was induced by subcutaneous injection of 10 ml/kg of 20%(w/v) brewer's yeast suspension at the dorsum region of each rat. After 17 hours of brewer's yeast injection, the temperature of each rat was measured using a digital thermometer by inserting the probe into the rectum 2 cm. Only the rat that showed an increase in temperature of at least 0.7°C was used for the experiment. After 30 min of treatment, the distilled water (10 ml/kg p.o.), aspirin (200 mg/kg p.o.) and the plant extract at doses of 50, 100 and 200 mg/kg p.o were given to the second, third, fourth and fifth group, respectively. The rectal temperature was measured at 1, 2, 3, 4 and 5 hours

### 2.8. Statistical analysis

The data were expressed as mean  $\pm$  S.E.M. and statistically analyzed using Student's *t*-test (independent) or one-way ANOVA followed by Bonferroni's test. *P* value less than 0.05 ( $P < 0.05$ ) was considered as significant different.



### 3. Results

#### 3.1 Acute toxicity test

The methanol extract of *P. sarmentosum* leaves at doses of 5 g/kg p.o. given to mice (10 males and 10 females of each group) did not affect behavioral responses during the observation period of 8 hours and 7 days after administration. No mortality was observed up to 7 days of monitoring. The LD<sub>50</sub> value of the extract in mice was estimated to more than 5 g/kg p.o. The extract at dose of 200 mg/kg p.o., the highest dose used in the present study was less than 25 fold when compared to the dose of 5 g/kg p.o. used in acute toxicity test. Therefore, the extract at doses of 50, 100 and 200 mg/kg p.o. given to mice or rats in this study was assumed to be safety.

#### 3.2 Analgesic activity

##### 1) Hot plate and tail flick response

Only the extract at dose of 200 mg/kg p.o. was found to significantly decrease the latency time of responses in experimental animals after 60 and 45 min post-administration, respectively compared to control. Morphine (5 mg/kg s.c.), a centrally acting analgesic drug, markedly increased pain latency both in hot plate and tail flick tests. Naloxone (2 mg/kg i.p.) given before morphine (5 mg/kg s.c.) or the extract (200 mg/kg p.o.) could antagonize the latency of nociceptive responses both in hot plate and tail flick tests (Table 1 and Table 2).

**Table 1**

Effects of the methanol extract of *Piper sarmentosum* leaves (MEPS), morphine and naloxone on the nociceptive response induced by the hot plate test in mice.

Treatment (N=10)	Dose (mg/kg)	Latency of nociceptive response (second)				
		30 min	45 min	60 min	75 min	90 min
Control (Distilled water) p.o.		8.57±0.33	8.72±0.22	8.70±0.31	9.14±0.24	8.47±0.31
Morphine sulfate s.c.	5	13.07±0.72**	18.68±0.76**	23.30±0.56**	26.32±0.50**	20.55±0.24**
Naloxone i.p.	2	8.42±0.37	8.63±0.28	8.68±0.33	8.95±0.24	8.92±0.29
MEPS p.o.	50	8.25±0.22	8.70±0.16	8.74±0.26	9.08±0.21	8.60±0.31
MEPS p.o.	100	8.33±0.38	8.62±0.24	8.74±0.13	8.86±0.40	8.89±0.30
MEPS p.o.	200	8.28±0.27	8.74±0.25	10.23±0.27*	10.68±0.33*	9.88±0.37*
Naloxone i.p.	2					
+ Morphine s.c.	5.	9.31±0.53 <sup>a</sup>	9.83±0.60 <sup>a</sup>	9.74±0.64 <sup>a</sup>	9.28±0.50 <sup>a</sup>	8.92±0.40 <sup>a</sup>
Naloxone i.p.	2					
+ MEPS p.o.	200	8.99±0.31	9.34±0.56	9.15±0.36 <sup>#</sup>	8.97±0.34 <sup>##</sup>	9.28±0.37

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

<sup>a</sup> $p < 0.01$ , significantly different compared to morphine (Independent  $t$ -test).

<sup>#</sup> $p < 0.05$ , <sup>##</sup> $p < 0.01$ , significantly different compared to MEPS 200 mg/kg (Independent  $t$ -test)

**Table 2**

Effects of the methanol extract of *Piper sarmentosum* leaves (MEPS), morphine and naloxone on the nociceptive response induced by the tail flick response in rats.

Treatment (N = 10)	Dose (mg/kg)	Latency of nociceptive response (second)				
		30 min	45 min	60 min	75 min	90 min
Control (Distilled water) p.o.		1.63±0.04	1.69±0.04	1.69±0.03	1.72±0.06	1.71±0.06
Morphine sulfate s.c.	5	2.50±0.07**	4.24±0.07**	4.46±0.07**	3.60±0.07**	3.23±0.08**
Naloxone i.p.	2	1.63±0.05	1.70±0.03	1.71±0.04	1.68±0.03	1.73±0.05
MEPS p.o.	50	1.73±0.03	1.69±0.05	1.65±0.03	1.80±0.07	1.76±0.05
MEPS p.o.	100	1.75±0.06	1.73±0.05	1.78±0.04	1.76±0.07	1.79±0.06
MEPS p.o.	200	1.83±0.05	1.94±0.07*	1.95±0.06**	2.04±0.07*	2.02±0.06
Naloxone i.p.	2					
+ Morphine s.c.	5	1.65±0.03 <sup>a</sup>	1.61±0.03 <sup>a</sup>	1.71±0.04 <sup>a</sup>	1.68±0.04 <sup>a</sup>	1.63±0.05 <sup>a</sup>
Naloxone i.p.	2					
+ MEPS p.o.	200	1.77±0.08	1.71±0.05 <sup>#</sup>	1.73±0.05 <sup>#</sup>	1.76±0.04 <sup>##</sup>	1.78±0.05 <sup>##</sup>

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

<sup>a</sup> $p < 0.01$ , significantly different compared to morphine (Independent  $t$ -test)

<sup>#</sup> $p < 0.05$ , <sup>##</sup> $p < 0.01$ , significantly different compared to MEPS 200 mg/kg (Independent  $t$ -test)

## 2) Acetic acid-induced writhing response

The extract at doses of 50, 100 and 200 mg/kg p.o. significantly reduced the number of writhings by 12.38, 24.02 and 31.24%, respectively compared to the reduction of 61.37% by 200 mg/kg p.o. aspirin (Table 3 and Fig. 1).

**Table 3**

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.

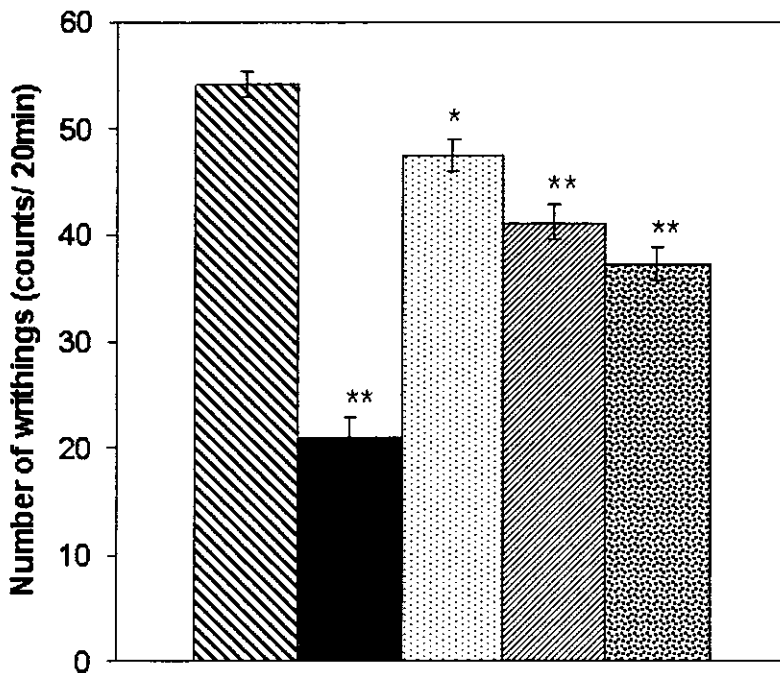
Treatment	Dose(mg/kg)	Number of writhings	Inhibition (%)
Control (DW 10ml/kg)		54.1±1.2	0
Aspirin	200	20.9±1.9**	61.37
MEPS	50	47.4±1.5*	12.38
MEPS	100	41.1±1.6**	24.02
MEPS	200	37.2±1.6**	31.24

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

Thirty minutes after treatment, mice were injected i.p. with 0.6% (v/v) acetic acid.

Number of writhings measured in a 20-minute period after i.p. injection of 0.6% (v/v) acetic acid

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



**Fig. 1.** Effects of the methanol extract of *Piper sarmentosum* leaves (MEPS) and aspirin on the nociceptive response induced by 0.6% acetic acid in mice (writhing test).

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

Symbols: Control (diagonal lines), Aspirin 200 mg/kg p.o. (solid black), MEPS 50 mg/kg p.o. (dotted),

MEPS 100 mg/kg p.o. (diagonal lines), MEPS 200 mg/kg p.o. (stippled)

### 3) Formalin test

Only the extract at dose of 100 mg/kg p.o. significantly reduced the licking activity only in the late phase. In contrast, morphine (5 mg/kg s.c.), aspirin (200 mg/kg p.o.) and higher dose of the extract (200 mg/kg p.o.), significantly decreased the licking activity both in early and late phase after given of a 20  $\mu$ l of 2.5% formalin, s.c. (Table 4 and Fig. 2).

**Table 4**

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

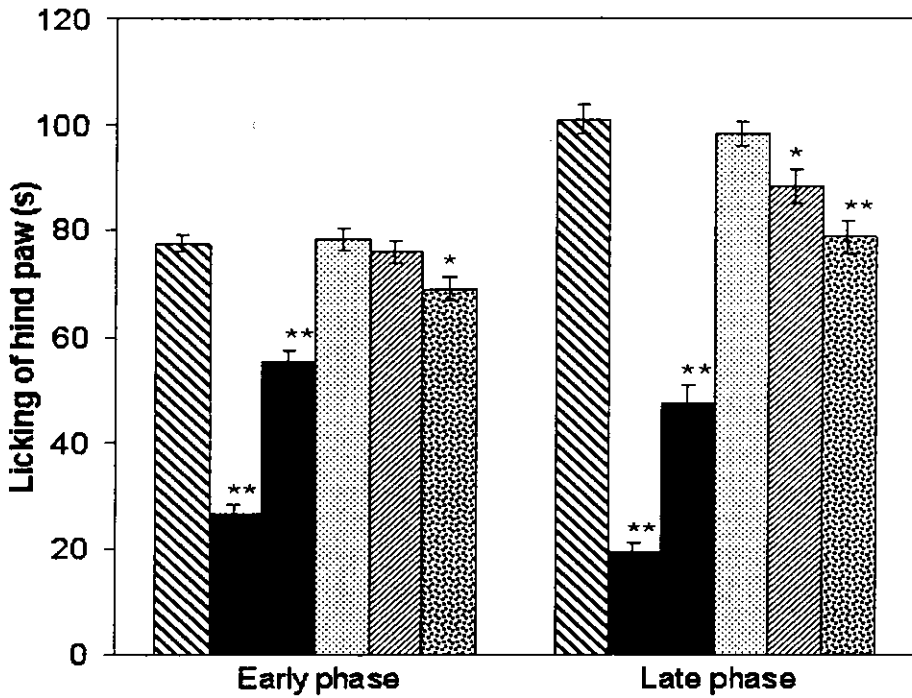
Treatment	Dose(mg/kg)	Licking of the hind paw (s)			
		Early phase (0-5 min)	Inhibition (%)	Late phase (15-30 min)	Inhibition (%)
Control (DW 10 ml/kg)		77.34 $\pm$ 1.52	0	100.81 $\pm$ 2.63	0
Morphine	5, s.c.	26.65 $\pm$ 1.87 <sup>**</sup>	65.54	19.30 $\pm$ 1.71 <sup>**</sup>	80.86
Aspirin	200	55.32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

Values are presented as mean  $\pm$  S.E.M. (N=10)

Thirty minutes after treatment, mice were injected s.c. to hind paw with 2.5% (v/v) formalin

Fifteen minutes after morphine, mice were injected s.c. to hind paw with 2.5% (v/v) formalin

<sup>\*</sup>  $p < 0.05$ , <sup>\*\*</sup>  $p < 0.01$ , significantly different compared with control (Bonferroni's test).



**Fig. 2.** Effects of the methanol extract of *Piper sarmentosum* leaves (MEPS) and aspirin on the nociceptive response (licking hind paw) induced by the formalin test in mice. \* $p < 0.05$ , \*\* $p < 0.01$ , significantly different compared to control (Bonferroni's test) Symbols: Control (▨), Morphine 5 mg/kg s.c. (■), Aspirin 200 mg/kg p.o. (■), MEPS 50 mg/kg p.o. (●), MEPS 100 mg/kg p.o. (▩), MEPS 200 mg/kg p.o. (⊞)

### 3.3 Anti-inflammatory activity

The extract at doses of 50, 100 and 200 mg/kg p.o. exhibited a significant inhibition of the rat paw edema induced by carrageenan. The extract at dose of 200 mg/kg exhibited an inhibition of 24.7% comparable to that of inhibition by 200 mg/kg p.o. of aspirin (33%) at the time 3 hours (Table 5 and Fig. 3).

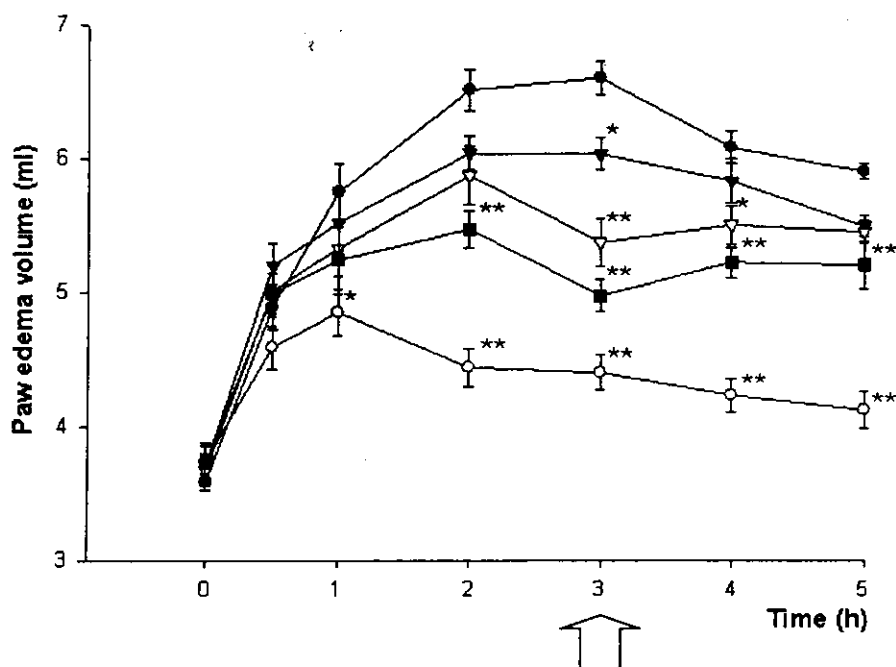
**Table 5** Effects of the methanol extract of *Piper sarmentosum* leaves (MEPS) and aspirin on carrageenan-induced paw edema in rats.

Treatment	Dose (mg/kg)	Initial paw volume (ml)	Paw edema volume (ml)					Inhibition of paw edema (%)						
			0.5 h	1 h	2 h	3h	4 h	5 h	1 h	2h	3 h	4 h	5 h	
Control (DW 10 ml/kg)		3.58±0.06	4.89±0.17	5.75±0.21	6.51±0.16	6.60±0.12	6.08±0.12	5.90±0.06						
Aspirin	200	3.74±0.14	4.57±0.16	4.85±0.17*	4.44±0.14**	4.40±0.13**	4.23±0.13**	4.12±0.14**	15.6	31.8	33.3	30.4	30.2	
MEPS	50	3.71±0.14	5.20±0.17	5.52±0.21	6.04±0.12	6.03±0.12*	5.83±0.17	5.49±0.08	4.0	7.2	8.6	4.1	7.0	
MEPS	100	3.67±0.12	5.01±0.19	5.33±0.21	5.87±0.22	5.37±0.18**	5.50±0.14*	5.45±0.08	7.3	9.8	18.6	9.5	7.6	
MEPS	200	3.73±0.12	4.99±0.15	5.24±0.26	5.74±0.14**	4.97±0.12**	5.22±0.11**	5.20±0.18**	8.9	11.8	24.7	14.1	11.9	

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

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





**Fig. 3.** Effects of the methanol extract of *Piper sarmentosum* leaves (MEPS) and aspirin on the carrageenan-induced paw edema in rats.

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

Symbols: Control (—●—), Aspirin 200 mg/kg p.o. (—○—), MEPS 50 mg/kg p.o. (—▼—), MEPS 100 mg/kg p.o. (—▽—), MEPS 200 mg/kg p.o. (—■—)

### 3.4 Antipyretic activity

The methanol extract of *P. sarmentosum* leaves (50, 100 and 200 mg/kg p.o.) did not reduce pyrexia induced by brewer's yeast in rats. The standard drug aspirin at dose of 200 mg/kg p.o. showed a significant antipyretic activity by causing a reduction in yeast-induced fever (Table 6 and Fig. 4).

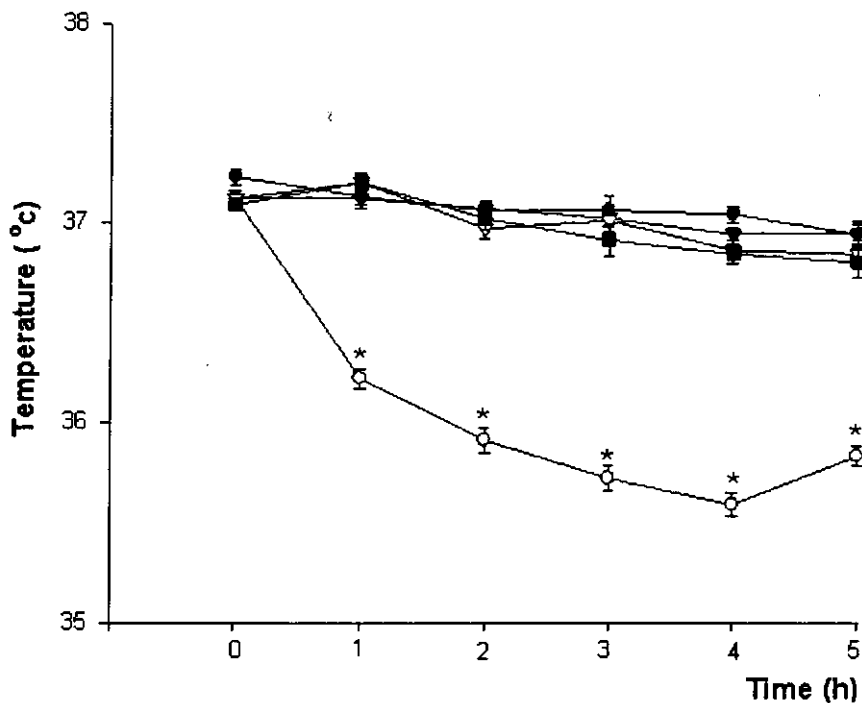
**Table 6** Effects of the methanol extract of *Piper sarmentosum* leaves (MEPS) and aspirin on the brewer's yeast induced pyrexia in rats.

Treatment	Dose (mg/kg)	Rectal temperature (° C)						
		Before yeast injection	Time after treatment (h)					
			0 h	1 h	2 h	3 h	4 h	5 h
Control (DW 10 ml/kg)		36.26±0.03	37.23±0.04	37.13±0.04	37.06±0.04	37.06±0.07	37.04±0.04	36.94±0.05
Aspirin	200	36.28±0.02	37.12±0.04	36.22±0.05*	35.91±0.06*	35.72±0.06*	35.59±0.06*	35.83±0.05*
MEPS	50	36.28±.03	37.12±0.04	37.12±0.05	37.07±0.04	37.02±0.04	36.94±0.03	36.94±0.07
MEPS	100	36.30±0.02	37.12±0.04	37.20±0.04	36.97±0.05	37.01±0.06	36.86±0.05	36.84±0.07
MEPS	200	36.29±0.02	37.09±0.03	37.20±0.05	37.02±0.06	36.91±0.08	36.84±0.05	36.80±0.08

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

Rectal temperature measured after yeast injection 17 h

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



**Fig. 4.** Effects of the methanol extract of *Piper sarmentosum* leaves (MEPS) and aspirin on the brewer's yeast induced pyrexia in rats.

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

Symbols: Control (●), Aspirin 200 mg/kg p.o. (○), MEPS 50 mg/kg p.o. (▼),

MEPS 100 mg/kg p.o. (▽), MEPS 200 mg/kg p.o. (■)

#### 4. Discussion

The methanol extract of *P. sarmentosum* leaves given by oral route in experimental animals exhibited the analgesic and anti-inflammatory activities in all tests used but lacked of an antipyretic effect in yeast-induced fever (Table 1-2, and Fig. 1-4). The LD<sub>50</sub> value of the extract given orally was estimated to be more than 5 g/kg in acute toxicity test. The standard drugs, morphine (5 mg/kg s.c.) and aspirin (200 mg/kg p.o.) were used for comparable to that of analgesic, anti-inflammatory and antipyretic activities of the extract.

The overall results suggested that the methanol extract of *P. sarmentosum* leaves exhibited the analgesic activity in all four tests used (hot plate, tail flick, writhing and formalin tests). It could be assumed that the extract had an analgesic activity mediated centrally acting in the central nervous system at levels of supraspinal and spinal. The hot plate assay is widely used for the evaluation of supraspinal analgesia [19]. The analgesic property is also studied using a sensitive model that thermal stimulus causes noxious stimuli on C fibers. In present study, the thermal stimuli was selected because of several advantages including sensitivity to a strong analgesics and limited tissue damages [20] and suitable for identification of centrally, but not peripherally acting analgesic drugs [21]. Sedatives and muscle relaxants may impair the ability to respond and hence be wrongly considered to have analgesic activity [13]. In addition, the tail flick assay is also widely used for evaluation of spinal analgesia [19]. It was a confirmation test for centrally acting analgesic compounds [22]. The drug-induced lengthening of the latency for the spinally mediated tail flick reflex is a reliable indicator of opioid-like antinociceptive activity [23]. Moreover, the centrally mediated analgesic action of the extract was confirmed using naloxone, a spinal antagonist of morphinomimetic receptor [24].

The results revealed that the analgesic action of the methanol extract of *P. sarmentosum* leaves was blocked by naloxone. Therefore, the extract showed analgesic activity through centrally mediated via supraspinal and spinal mechanisms similar to that of morphine. The acetic acid-induced writhing response is used as a chemical stimulus to induce the writhing syndrome and cause algia by releasing of endogenous substances including serotonin, histamine, bradykinin, substance P and prostaglandins, which then excite the pain nerve endings to cause the abdominal writhing. This test is a visceral pain model [25]. The writhing test is used for screening of peripherally and centrally acting analgesic activities [26]. The quantification of prostaglandins by radioimmunoassay in the peripheral exudates of mice obtained after the intraperitoneal injection of acetic acid demonstrated high level of prostaglandin E<sub>2</sub> and F<sub>2α</sub> during 30 min after stimulus [27]. In our study, the extract exhibited an analgesic effect in the writhing model, suggested that this effect was also mediated by peripheral mechanisms via the inhibition of prostaglandin synthesis. The formalin assay is an another pain model which assesses the way of an animal to respond to moderate, continuous pain generated by injured tissues [28]. This test possesses 2 distinctive phases, the early phase reflects a direct effect of formalin on nociceptors via a central effect (neurogenic pain) and the late phase reflects inflammatory pain via peripheral effect [29,30]. The methanol extract of *P. sarmentosum* leaves (200 mg/kg p.o.) produced a significant reduction of licking activity both in early and late phase, suggested that the extract had an analgesic action peripherally and centrally mediated mechanisms via inhibition of the synthesis of prostaglandins. In addition to its antinociceptive action, the extract may also possess the anti-inflammatory activity at the same time due to the inhibition of prostaglandin synthesis.

The carrageenan-induced rat paw inflammation is useful for investigation of the systemic anti-inflammatory activity of drugs. This model is commonly used as an experimental animal model for acute inflammation and sensitive to most clinically effective anti-inflammatory drugs. Edema formation due to carrageenan in the rat paw is biphasic events; the initial phase (1-2 hours) is mediated via the release of cytoplasmic enzymes, prostaglandins, histamine and bradykinin whereas the second phase (3-5 hours) was involved in the release of superoxide, prostaglandins, leukotrienes and bradykinin. The latter phase is sensitive to most clinically effective anti-inflammatory drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs)[31-34]. The results showed that the extract significantly reduced the rat paw edema volume induced by carrageenan in the second phase, suggested that it possessed an anti-inflammatory activity. The standard drug aspirin markedly decreased the rat paw edema volume induced by carrageenan both in the initial and second phase. Therefore, the methanol extract of *P. sarmentosum* leaves is likely to possess the anti-inflammatory activity similar to that of aspirin (Fig. 3).

Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, mainly PGE<sub>2</sub>, which increase set point for thermoregulation to higher temperature. The inhibition of prostaglandin synthesis could be the possible mechanism of an antipyretic action such as acetylsalicylic acid [35]. The extract (50, 100 and 200 mg/kg p.o.) had no a significant reduction of yeast-induced fever (Fig. 4).

In conclusion, the overall results suggested that the methanol extract of *P. sarmentosum* leaves showed an analgesic activity mediated peripherally and centrally on nervous system pathways. These mechanisms are likely to act as morphine, a standard analgesic drug. The extract also exhibited the anti-inflammatory activity

which mechanisms of action are most likely to aspirin, a prototype of NSAIDs. However, in the present study it lacks of antipyretic activity. The present results of the analgesic and anti-inflammatory activities of the methanol extract of *P. sarmentosum* leaves seems to support the traditional use of this plant in folk lore medicine. The further studies would be done concerning to isolation, identification of an active component in *P. sarmentosum*, and testing for its analgsic, anti-inflammatory and antipyretic activities since many biological active compounds are found in various parts of *P. sarmentosum*.

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