

Studies of the anti-inflammatory and antipyretic activities of the methanolic extract of *Piper sarmentosum* Roxb. leaves in rats

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Abstract

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The methanolic extract of *Piper sarmentosum* Roxb. leaves at doses of 50, 100 and 200 mg/kg was investigated for anti-inflammatory and antipyretic activities on carrageenan-induced rat paw edema and brewer's yeast-induced pyrexia in rats. The results revealed that the extract at test doses produced a significant anti-inflammatory activity at 3 h with an inhibition of paw edema of 8.6% ($P<0.05$), 18.6% ($P<0.01$) and 24.7% ($P<0.01$), respectively, compared to the reference drug aspirin 200 mg/kg p.o. with an inhibition of 33.3% ($P<0.01$). Only the extract at the dose of 200 mg/kg p.o. showed a significant inhibition of carrageenan-induced rat paw edema beginning at 2 h of 11.8% ($P<0.01$) and at 3, 4 and 5 h of 24.7% ($P<0.01$), 14.1% ($P<0.01$) and 11.9% ($P<0.01$), respectively, whereas the reference drug aspirin 200 mg/kg p.o. exhibited a significant inhibition of edema beginning at 1 h of 15.6% ($P<0.05$) and at 2, 3, 4 and 5 h of 31.8%

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($P < 0.01$), 33.3% ($P < 0.01$), 30.4% ($P < 0.01$) and 30.2% ($P < 0.01$), respectively. The methanolic extract of *P. sarmentosum* leaves (50, 100 and 200 mg/kg p.o.) did not decrease brewer's yeast-induced pyrexia in rats, whereas aspirin at the dose of 200 mg/kg p.o. showed a significant antipyretic activity by reducing fever in this animal model. In acute toxicity test, the methanolic extract of *P. sarmentosum* leaves at the dose of 5 g/kg did not produce any abnormal symptoms or mortality in rats.

Key words : *Piper sarmentosum* leaves, anti-inflammatory activity, antipyretic activity, methanolic extract

บทคัดย่อ

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การศึกษาฤทธิ์ต้านการอักเสบและลดไข้ของสารสกัดเมธานอลจากใบชะพลูในหนูขาว
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งานวิจัยนี้ศึกษาฤทธิ์ต้านการอักเสบและลดไข้ของสารสกัดเมธานอลจากใบชะพลูในขนาด 50, 100 และ 200 มก./กก. ที่บริหารโดยการป้อนทางปากแก่หนูขาวที่เหนียวทำให้ไข้สูงเข้ามด้วยคาร์ราจีแนหรือทำให้เกิดไข้ด้วยยีสต์ ผลการทดลอง พบว่า สารสกัดเมธานอลจากใบชะพลูในขนาดที่ใช้ในการทดสอบมีฤทธิ์ต้านการอักเสบอย่างมีนัยสำคัญที่เวลา 3 ชั่วโมงโดยสามารถยับยั้งอาการบวมของอุ้งเท้าหนูได้ 8.6%, 18.6% และ 24.7% ตามลำดับ เมื่อเปรียบเทียบกับยาแอสไพรินในขนาด 200 มก./กก. ที่บริหารโดยการป้อนทางปากซึ่งสามารถยับยั้งอาการบวมของอุ้งเท้าหนูได้ 33.3% สารสกัดเมธานอลจากใบชะพลูในขนาด 200 มก./กก. เท่านั้นที่สามารถยับยั้งอาการบวมของอุ้งเท้าหนูอย่างมีนัยสำคัญที่เวลา 2, 3, 4 และ 5 ชั่วโมงได้ 11.8%, 24.7%, 14.1% และ 11.9% ตามลำดับ ในขณะที่ยาแอสไพรินในขนาด 200 มก./กก. สามารถยับยั้งอาการบวมของอุ้งเท้าหนูได้ 15.6% ที่เวลา 1 ชั่วโมงและที่เวลา 2, 3, 4 และ 5 ชั่วโมงลดอาการบวมของอุ้งเท้าหนูได้ 31.8%, 33.3%, 30.4% และ 30.2% ตามลำดับ สารสกัดเมธานอลจากใบชะพลูในขนาดที่ใช้ในการทดลองไม่มีฤทธิ์ในการลดไข้ที่เกิดจากการเหนียวของยีสต์ในหนูขาวเมื่อเปรียบเทียบกับยาแอสไพรินในขนาด 200 มก./กก. ซึ่งมีฤทธิ์ในการลดไข้ในหนูขาวที่เกิดจากการเหนียวของยีสต์ได้ในการทดสอบความเป็นพิษของสารสกัดเมธานอลจากใบชะพลู พบว่าสารสกัดเมธานอลจากใบชะพลูในขนาด 5 กรัม/กก. เมื่อป้อนทางปากไม่ทำให้หนูขาวมีอาการผิดปกติหรือตายแต่อย่างใด

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Piper sarmentosum Roxb. (Piperaceae), commonly known as "Cha Plu" in Thai, is a plant widely distributed throughout every region in Thailand (Suvatti, 1978). The plant is a terrestrial herb with 60 cm height, green trunk and jointed at the nodes. The leaves are thin, dark green, heart shaped and spicy tasted. According to traditional medicine in Thailand, this plant has been used as an expectorant, carminative and antispasmodic, for refreshing the throat, antiflatulence, and enhancing appetite, and to relieve asthma, muscle pains and cough (Pongboonrod, 1976; Ning-Hon, 1980;

Apisariyakul, 1984; Apisariyakul and Anantasarn, 1984). In Malaysian and Indonesian traditional medicine, the plant is used for toothache, fungoid dermatitis on the feet, coughing asthma, pleurisy, diabetes, hypertension and joint aches (Perry, 1981; Subramaniam et al., 2003). The previous pharmacological studies of *Piper sarmentosum* extract showed antimicrobial activity against *Escherichia coli* and *Bacillus subtilis* (Matsuda et al., 1991), hypoglycemic effect (Peungvicha et al., 1998; Pongmarutai, 1980), neuromuscular blocking activity in rat phrenic nerve-hemidiaphragm

preparation (Ridtitid *et al.*, 1998), antiplasmodial activity against *Plasmodium falciparum* and *Plasmodium berghei* (Rahman *et al.*, 1999), antioxidant activity (Subramaniam *et al.*, 2003) and antiprotozoal effect against *Entamoeba histolytica* (Swangchareon *et al.*, 2004). Recently, many medicinal plant species used in traditional medicine have been investigated in experimental animals for anti-inflammatory, antinociceptive and antipyretic activities, for example, *Clerodendrum petasites* (Panthong *et al.*, 2003), *Diospyros variegata* (Trongsakul *et al.*, 2003), *Ventilago harmandiana* (Panthong *et al.*, 2004), *Aegle marmelos* (Arul *et al.*, 2005) and *Phrygilanthus acutifolius* (Daud *et al.*, 2006) and revealed that all possess these effects.

Previous studies have shown that various parts of this plant contain many biologically active compounds such as asaricin, α -asarone (Matsuda *et al.*, 1991), hydrocinnamic acid, β -sitosterol (Niamsa and Chanpromma, 1983), sarmentine, sarmentosine (Likhitwitayawuid *et al.*, 1987), vitamin C, E and carotenes (Chanwitheesuk *et al.*, 2004), longifolene, β -caryophyllene, allo-aromadendrene, 9-epi-(E)-caryophyllene, β -asarone, viriflorene and β -selinene (Aunpak *et al.*, 1997), guineensine, brachystamide B, brachyamide B, sesamin, 1-piperrettyl pyrrolidine, 3',4',5'-trimethoxycinnamoyl pyrrolidine, (+)-asarinin and methyl piperate (Rukachaisirikul *et al.*, 2004). In folk medicine, the plant was applied as a remedy for toothache, headache, asthma and joint aches, and to reduce fever in influenza patients (Perry, 1981; Aunpak *et al.*, 1997; Subramaniam *et al.*, 2003; Rukachaisirikul *et al.*, 2004). According to use in traditional medicine, this plant was primarily expected to possess anti-inflammatory, antinociceptive and antipyretic activities. Therefore, the present work was undertaken to investigate the anti-inflammatory and antipyretic activities of the methanolic extract of *P. sarmentosum* leaves to verify the claimed curative properties which have not been scientifically proven in herbal medicine, and to explain possible mechanisms of the methanolic extract of *P. sarmentosum* leaves in comparison with the reference drug aspirin. The

study of antinociceptive activity is not included in this investigation.

Materials and Methods

Plant material

Fresh leaves of *P. sarmentosum* Roxb. were collected in March, 2005 from Ranod district, Songkhla province, Thailand. The taxonomical identification of this plant was made 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.

Preparation of the extract and reference drug

Forty kilograms of 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 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 to give 480 ml of a black viscous and oil-like extract mixture. This extract mixture was lyophilized 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 methanolic extracts of *P. sarmentosum* leaves at doses of 50, 100 and 200 mg/kg were prepared by suspending in 0.9% normal saline. The reference drug used in this study was aspirin at the dose of 200 mg/kg which was prepared by dissolving in 0.9% normal saline.

Experimental animals

Male Wistar rats (140-170 g) were used for the experiment. The animals were obtained from the Southern Laboratory Animal Facility, Prince of Songkla University, Hat Yai, Songkhla, Thailand, and kept in a room with 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*.

Acute toxicity test

The 50% lethal dose (LD_{50}) of the methanolic extract of *P. sarmentosum* leaves was estimated by up-and-down method in mice (Bruce, 1985). 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 (10 mice of each set). 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 h and 7 days after administration. Food and water were given *ad libitum*.

Anti-inflammatory activity

The carrageenan-induced rat paw edema model was used for investigation of the anti-inflammatory activity (Winter *et al.*, 1962). In brief, 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 reference 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 groups, respectively. After 30 min of treatment, carrageenan was injected. The volume of hindpaw was measured at 0.5, 1, 2, 3, 4 and 5 h after carrageenan injection.

Antipyretic activity

Antipyretic activity was studied using the method previously described with a minor modification (Adam *et al.*, 1968). The groups of animal used were similar to those in testing of anti-inflammatory activity. Briefly, all rats were fasted overnight with water *ad libitum* before use. 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. Seventeen hours

after brewer's yeast injection, the temperature of each rat was measured using a digital thermometer by inserting the probe into the rectum at the depth of 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, all test drugs were given orally. The rectal temperature was measured at 1, 2, 3, 4 and 5 h.

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 a significant difference.

Results

Acute toxicity test

The methanolic extract of *P. sarmentosum* leaves at the dose 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 h and 7 days after administration. No mortality was observed up to 7 days of monitoring. The LD_{50} 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 25 fold less than the dose 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 safe.

Anti-inflammatory activity

The extract at doses of 50, 100 and 200 mg/kg p.o. produced a significant anti-inflammatory activity at 3 h with paw edema inhibition of 8.6% ($P < 0.05$), 18.6% ($P < 0.01$) and 24.7% ($P < 0.01$), respectively, while the reference drug aspirin (200 mg/kg p.o.) inhibited paw edema of 33.3% ($P < 0.01$). Only the extract at the dose of 200 mg/kg p.o. showed a significant inhibition of carrageenan-induced rat paw edema beginning at 2 h of 11.8% ($P < 0.01$) and at 3, 4 and 5 h of 24.7% ($P < 0.01$), 14.1% ($P < 0.01$) and 11.9% ($P < 0.01$), respectively, whereas the reference drug aspirin

200 mg/kg p.o. exhibited a significant inhibition of edema beginning at 1 h of 15.6% ($P<0.05$) and at 2, 3, 4 and 5 h of 31.8% ($P<0.01$), 33.3% ($P<0.01$), 30.4% ($P<0.01$) and 30.2% ($P<0.01$), respectively. At 3 h the extract at the dose of 200 mg/kg exhibited inhibitory effect on carrageenan-induced rat paw edema comparable to that of aspirin at the dose of 200 mg/kg (Table 1).

Antipyretic activity

The methanolic 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 reference drug aspirin at the dose of 200 mg/kg p.o. showed a significant antipyretic activity by reducing yeast-induced fever in rats (Table 2).

Discussion and Conclusion

The methanolic extract of *P. sarmentosum* leaves at doses of 50, 100 and 200 mg/kg given by oral route in experimental animals exhibited marked anti-inflammatory activity, comparable to the reference drug aspirin (200 mg/kg p.o.), a prototype of non-steroidal anti-inflammatory drugs (NSAIDs). However, in the present investigation, the methanolic extract at test doses lacked of an antipyretic effect in brewer's yeast-induced fever. The LD₅₀ value of the extract given orally was estimated to be more than 5 g/kg in mice.

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 because of its sensitivity in detecting orally active anti-inflammatory agents particularly in the acute phase of inflammation (DiRosa *et al.*, 1971; DiRosa, 1972). The test is sensitive to most clinically effective anti-inflammatory drugs. The intraplantar injection of carrageenan in rats leads to paw edema. Edema formation due to carrageenan injection in the rat paw is biphasic events; the initial phase (0-2.5 h after carrageenan injection) results from the concomitant release of mediators: histamine, serotonin and kinins on the vascular permeability (Daud *et al.*, 2006) while the second

Table 1. Effects of the methanolic 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	3 h	4 h	5 h	1 h	2 h	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).

Table 2. Effects of the methanolic 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±0.03	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).

phase is associated with the increased production of inducible cyclooxygenase (COX) and thereby increases the synthesis of prostaglandins, as well as the production of oxygen-derived free radicals (Panthong *et al.*, 2004). The COX products, particularly prostaglandin E₂ (PGE₂), contribute to increased blood flow through a vasodilatation action, but the lipoxygenase pathway is necessary for vascular leakage and edema consequent on cellular infiltration (Wedmore and Williams, 1981). The second phase is sensitive to most clinically effective anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) (Vinegar *et al.*, 1969, 1987; DiRosa *et al.*, 1971; Crunkhon and Meacock, 1971). Our results indicated that oral administration of test doses of the methanolic extracts of *P. sarmentosum* significantly reduced the rat paw edema volume at 3 h after carrageenan injection except the dose of 200 mg/kg still continued to exhibit this activity after 3 h, suggesting that the extract possessed an anti-inflammatory activity. The onset of anti-inflammatory action of all test doses occurred at 3 h after oral administration. This delayed onset of anti-inflammatory action of the extract is probably involved with alterations in pharmacokinetic processes i.e., slow absorption of active component (s) from the rat gastrointestinal tract or undergoing gastrointestinal inactivating metabolism, which needs to be further studied. The reference drug aspirin markedly decreased the rat paw edema

volume induced by carrageenan both in the initial and second phase. Aspirin, a nonselective inhibitor of both cyclooxygenase isoforms (COX 1 and COX 2), exhibits anti-inflammatory action by inhibiting prostaglandins, thromboxane and prostacyclin synthesis involved in inflammation (Katzung, 2004). Furthermore, aspirin also interferes with the chemical mediators of the kallikrein-kinin system (kallikreins and kinins), thus inhibiting granulocyte adherence to damaged vasculature, stabilizing lysosomes, and inhibiting the chemotaxis of polymorphonuclear leukocytes and macrophages (Katzung, 2004). Kinins play an important role in the inflammatory process. Kallikreins and kinins can produce redness, local heat, swelling, and pain, and the production of kinins is increased in inflammatory lesions produced by a variety of methods (Katzung, 2004). When these data are taken together, we could conclude that the anti-inflammatory effect of the methanolic extract of *P. sarmentosum* leaves might relate to inhibition of mediators that corresponds to its action on each phase of inflammation similar to that of aspirin.

Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, mainly PGE₂, which raise the set point for thermoregulation to a higher temperature. Aspirin reduces elevated temperature, and its antipyretic effect is probably mediated by both COX inhibition in the central nervous system and inhibition of interleukin-1 (IL-1) which is released

from macrophages during episodes of inflammation (Katzung, 2004). The inhibition of prostaglandin synthesis could be the possible mechanism of an antipyretic action such as aspirin (Howard, 1993). In the present study, the methanolic extract of *P. sarmentosum* at doses of 50, 100 and 200 mg/kg p.o. did not produce significant reduction of yeast-induced fever.

In conclusion, from the evidence of this study, we suggest that the methanolic extract of *P. sarmentosum* leaves possesses anti-inflammatory activity while lacking antipyretic activity. The anti-inflammatory action of the methanolic extract of *P. sarmentosum* leaves might relate to inhibition of mediators involved in each phase of inflammation. However, we could assume that the anti-inflammatory activity of the extract is most likely similar to aspirin, a prototype of NSAIDs. The anti-inflammatory activity of the methanolic extract of *P. sarmentosum* leaves found in this investigation seems to support the traditional use of this plant in folklore medicine. Further studies concerning the isolation and identification of active component (s) in *P. sarmentosum* and testing for its anti-inflammatory activity would be useful.

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