Anti-Inflammatory, Anti-Nociceptive and Antipyretic Effects of the Ethanol Extract From Root of *Piper Sarmentosum* Roxb.

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Background: *Piper sarmentosum Roxb. (Cha Phul) is a plant in the Piperaceae family which the whole plant is used as an expectorant and the leaf as a carminative. Many extracts from the plants in this family show anti-nociceptive, anti-inflammatory and antipyretic activities in various animal models.*
Objective: *To investigate the anti-inflammatory, anti-nociceptive and antipyretic effects of the ethanol extract from P. sarmentosum root.*
Material and Method: *In vivo study.*
Results: *P. sarmentosum extract significantly inhibited ethyl phenylpropiolate-induced ear edema as well as carrageenan-induced hind paw edema in rats. The extract reduced transudative and granuloma weights of the chronic inflammatory model using the cotton pellet-induced granuloma formation in rats. The extract exerted a pronounced inhibitory activity on the early phase and late phase of the formalin test in mice. In addition, the extract elicited an antipyretic activity on yeast-induced hyperthermia in rats.*
Conclusion: *P. sarmentosum extract possessed anti-inflammatory, anti-nociceptive and antipyretic activities.*

Keywords : *Piper sarmentosum, Anti-inflammatory, Anti-nociceptive, Antipyretic*

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Piper sarmentosum Roxb. (family Piperaceae) commonly known in Thailand as "Cha Phul", is native to India, Southern China and Southeast Asia. In Thai herbal traditional medicine, the whole plant is used as an expectorant, the leaf as a carminative. The pharmacological activities of *P. sarmentosum* extract have been reported in many studies. The water fruit extract exhibited antidiabetic[1]. Methanolic leave extract was exhibited neuromuscular blocking[2], antioxidant[3] and antibacterial[4] activities. Many extracts from plants of this family have been proven to possess antinociceptive, anti-inflammatory and antipyretic activities in many animal models. *n*-Hexane extract of aerial part of *P. sarmentosum* showed anti-inflammatory activity[5]. The purpose of this study was to evaluate the anti-inflammatory effect of the ethanol extract from root of *P. sarmentosum* in animal models. Other activities, *i.e.* anti-nociceptive and antipyretic activities were also examined.

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Material and Method

Plant materials

Roots of *P. sarmentosum* were collected from Chombueng, Ratchaburi, Thailand. Authentications of

plant materials were carried out at the herbarium of the Department of Forestry Bangkok, Thailand, where the herbarium voucher (SKP 146161901) were kept to specimen in the herbarium of Southern Center of Thai Medicinal plant at Faculty of Pharmaceutical Science, Prince of Songkhla University, Songkhla, Thailand.

Preparation of P. sarmentosum ethanolic extract (EPS extract)

Roots of *P. sarmentosum* were washed with water to remove the remaining sand and to reduce the microbial load. The cleaned plant material was cut into small pieces and dried at 50°C, powdered and extracted similar to that practiced by Thai traditional doctors. One kilogram of dried fruit material was macerated with 95% ethanol for 3 days, filtered and concentrated to dryness under pressure. The marc was macerated 2 times and dried by an evaporator. The percentage yield was 6.21 of raw material.

Experimental animals

Male Sprague Dawley rats weighing 40-60, 100-120 and 200-250 g as well as male ICR mice weighing 30-40 g were obtained from the National Laboratory Animal Center, Nakorn Pathom, Thailand. Animals were randomly assigned to control and treatment groups. They were kept in a room maintained under environmental conditions of 25 $\pm$ 1°C and 12 h dark-light cycle. The animals had free access to water and food. Rats were kept in experimental facility for 1 week to allow them to be acclimated prior to dosing. The Animal Ethics Committee of Faculty of Medicine, Thammasat University, Pathumthani, Thailand, approved all experimental protocols (No. 0002/2008).

Anti-inflammatory study

Ethyl phenylpropiolate (EPP)-induced ear edema in rats[6]

Male rats weighing 40-60 g were used. Ear edema was induced by topical application of EPP 1 mg/ 20 ml/ear to the inner and outer surface of both ears. The test substances were applied in the same manner in a volume of 20 ml just before the irritants. The thickness of each ear was measured with digital vernier calipers before and at 15, 30, 60 and 120 min after edema induction.

Carrageenan-induced paw edema in rats[7]

Male rats of 100-120 g body weight orally received the test substance 1 h prior to carrageenan injection. A volume of 0.05 ml of 1% carrageenan in sterile normal saline solution (NSS) was injected intradermally into the plantar side of the right hind paw of the rat. The edema volumes were determined using a plethysmometer (model 7140, Ugo Basile, Italy) prior to and 1, 3 and 5 h after carrageenan injection.

Cotton pellet-induced granuloma formation in rats[8]

Male rats of 200-250 g body weight were used. Two sterilized cotton pellets (20 $\pm$ 1 mg) were implanted subcutaneously, one on each side of the abdomen of the animal under thiopental anesthesia. Test drugs were administered orally in a once daily dosage regimen throughout the experimental period of 7 days whereas the control group receives distilled water only. On the 8th day after implantation, rats were anaesthetized with thiopental sodium. Both cotton pellets and thymus were dissected, dried at 60°C for 18 h and their dry weight determined. The change in body weight from the first and the last day of experiment was also recorded. The transudative and granuloma weight, as well as the percentage of granuloma inhibition of the test drugs were calculated.

Anti-nociceptive study

Formalin test in mice[9]

Male ICR mice weighing 30-40 g were used in the formalin test. Test substances were administered orally 1 h before the formalin injection, while morphine was administered intraperitoneal injection 30 min for the early phase assessment. The 20 ml of 1% formalin in NSS was injected subcutaneously into the left dorsal hind paw after test substance administration. The time mice spent in licking of the injected hind paw was determined between 0-5 min after formalin injection. In the late phase, the formalin was injected 40 min after oral administration except morphine (10 min) and the licking time is determined between 20-30 min after the injection of formalin.

Antipyretic study

Yeast-induced hyperthermia in rats[10]

Male rats weighing 200-250 g were used. Before pyrexia is induced, the initial rectal temperatures were recorded using a twelve channel electric thermometer (LETICA, model TMP 812 RS, Panlab SL, Spain). Thereafter hyperthermia was induced in rats by subcutaneous injection of 1 ml/100 g body weight of 25% yeast in NSS. When the temperature was at a peak, 18 h after yeast injection, the rectal temperatures were again recorded. Those animals which showed a rise in

rectal temperature of more than 1°C were used for the antipyretic test. Test substances were orally administered the animals and the rectal temperatures of animals were recorded at 30 min interval for 2 h following drug treatment.

Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). Statistical significance was determined by one-way analysis of variance (ANOVA) and post hoc least-significant difference (LSD) test. P-values less than 0.05 were considered significant.

Results

Effect of EPS extract on EPP-induced ear edema in rats

A topical application of EPP on rat ears produced a marked edema formation (Table 1). EPS extract at the dose of 1 mg/ear significantly inhibited the ear edema formation.

Effect of EPS extract on carrageenan-induced paw edema in rats

As shown in Fig. 1, EPS extract, at doses of 1,200 mg/kg markedly reduced the paw edema at 3 and 5 h after carrageenan injection. In the positive control group, aspirin (a cyclooxygenase inhibitor) at the dose of 300 mg/kg produced significant inhibitory effect of the paw edema at all assessment times.

Effect of EPS extract on cotton pellet-induced granuloma formation in rats

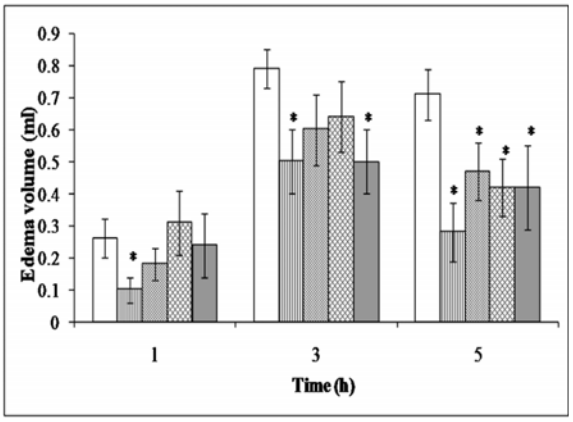
EPS extract at the dose of 1,200 mg/kg reduced only transudative weight whereas aspirin (300 mg/kg) did not reduce transudative weight and granuloma formation. The group treated with prednisolone (5 mg/kg, po) daily for 7 days elicited a marked inhibition on both parameters (Table 2). In addition, prednisolone significantly reduced the body weight gain and thymus weight of the animals whereas EPS extract and aspirin did not affect these parameters (data not shown).

Effect of EPS extract on formalin test in mice

All doses of EPS extract, aspirin and morphine significant elicited an inhibitory effect on the formalin test in mice (Fig. 2).

Effect of EPS extract on yeast-induced hyperthermia in rats

As shown in Table 3, all doses of EPS extract



(□ : control; ▥ : aspirin 300 mg/kg; ▧ : EPS 300 mg/kg; ▩ : EPS 600 mg/kg; ■ : EPS 1,200 mg/kg).

Fig. 1 Effect of EPS extract on carrageenan-induced paw edema. Significantly difference from control: *p < 0.05.

Table 1. Effect of EPS extract on ethyl phenylpropiolate (EPP)-induced ear edema

Group	Dose (mg/ear)	Time after topical application of EPP							
		15 min		30 min		60 min		120 min	
		ED (mm)	EDI (%)	ED (mm)	EDI (%)	ED (mm)	EDI (%)	ED (mm)	EDI (%)
Control	-	116.67 ± 11.74	-	191.67 ± 19.49	-	226.67 ± 26.92	-	95.00 ± 7.19	-
Phenyl-butazone	1	40.00 ± 15.71*	66	51.67 ± 21.36*	73	93.33 ± 25.91*	59	75.00 ± 12.58	21
EPS extract	1	25.00 ± 5.63*	78	58.33 ± 21.67*	69	110.00 ± 13.66*	51	81.67 ± 13.02	14

Data represent mean ± SEM (n = 6) *Significantly different from the control group, p < 0.05, ED: edema thickness; EDI: edema inhibition

Table 2. Effect of EPS extract on granuloma formation and transudation in cotton pellet-induced granuloma formation model

Group	Dose (mg/kg)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)	GI (%)
Control	-	518.38 ± 58.06	90.99 ± 8.24	427.39 ± 50.21	3.55 ± 0.41	-
Prednisolone	5	289.89 ± 10.76*	66.62 ± 4.69*	223.27 ± 8.65*	2.33 ± 0.23*	34
Aspirin	300	473.90 ± 25.69	99.20 ± 9.22	374.70 ± 22.61	3.96 ± 0.46	0
EPS extract	1,200	388.51 ± 28.45*	78.59 ± 5.21	309.92 ± 23.67*	2.93 ± 0.26	17

Data represent mean ± SEM (n = 6). *Significantly different from the control group, $p < 0.05$, GI = granuloma inhibition

Table 3. Effect of EPS extract on yeast-induced hyperthermia in rats

Group	Rectal temperature (°C)					
	Normal temp	18 h after yeast injection	Time after yeast injection			
			30 min	60 min	90 min	120 min
Control	37.68 ± 0.15	38.75 ± 0.18	38.97 ± 0.13	38.82 ± 0.15	38.73 ± 0.13	38.68 ± 0.13*
Aspirin 300 mg/kg	37.60 ± 0.12	38.70 ± 0.14	38.07 ± 0.14*	37.72 ± 0.14*	37.60 ± 0.14*	37.48 ± 0.13*
EPS extract						
300 mg/kg	37.42 ± 0.15	38.52 ± 0.10	37.95 ± 0.29*	37.65 ± 0.36*	37.72 ± 0.35*	37.67 ± 0.36*
600 mg/kg	37.48 ± 0.15	38.73 ± 0.14	37.90 ± 0.38*	37.72 ± 0.41*	37.62 ± 0.39*	37.62 ± 0.42*
1,200 mg/kg	37.57 ± 0.07	38.50 ± 0.10	37.48 ± 0.40*	37.95 ± 0.09*	37.82 ± 0.10*	37.63 ± 0.13*

Data represent mean ± SEM (n = 6). *Significantly different from the control group, $p < 0.05$.

and aspirin possessed a significant decrease in the rectal temperature of hyperthermia in rats at all recorded times.

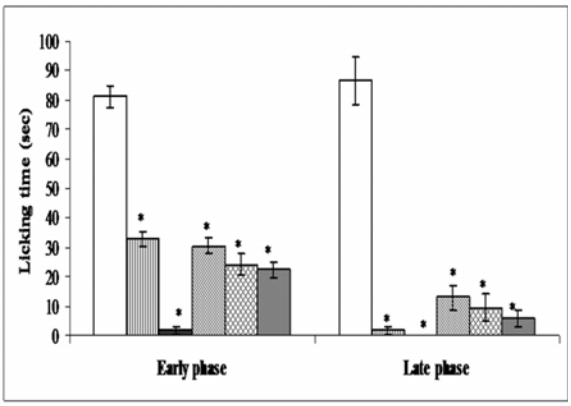


Fig. 2 Effect of EPS extract on formalin test. Significantly difference from control: *$p < 0.05$.

Discussion

The anti-inflammatory, analgesic and antipyretic activities of EPS extract were evaluated in the present study. EPP-induced rat ear edema is a screening model for investigating the anti-inflammatory activity of test substances on acute phase of inflammation. The inflammatory mediators, including histamine, serotonin, bradykinin and prostaglandin are typically released in this model. These mediators are capable for promoting vasodilatation, increasing vascular permeability, and producing edema[11]. EPS extract exhibited an inhibitory effect on the ear edema formation induced by EPP. These results suggest that EPS extract probably possesses anti-inflammatory activity by inhibiting the inflammatory mediators of the acute phase of inflammation.

The carrageenan test has been widely used for evaluating the anti-inflammatory agents particularly in the acute phase of inflammation[7]. The carrageenan injection in rats' paw leads to the paw edema. Inflammatory process involves three phases by several

mediators released in ordinate sequence. The first phase after carrageenan injection (0-1.5 h), results from the release of histamine and serotonin. The second phase (1.5-2.5 h) is mediated by bradykinin. The third phase (2.5-6 h) is correlated with the production of prostaglandins[12]. Oral administration of EPS extract suppressed the hind paw edema at the third and fifth hour, suggests that the main mechanism of action may involve the biosynthesis of prostaglandins and/or other inflammatory mediators.

Cotton pellet-induced granulation is commonly used as an *in vivo* test for chronic inflammation. The response to a subcutaneously implanted cotton pellet in rat is divided into at least three phases, transudative phase, exudative phase, and proliferative phase[8]. Moreover, the implanted material induces a host inflammatory response and modulates the release of inflammatory mediators which finally leads to tissue proliferation and granular formation[13-15]. Anti-inflammatory drugs can reduce transudative weight probably via their ability to inhibit the permeability response of the blood vessels around the cotton pellet implantation. They can also effectively inhibit the granuloma formation probably due to interference with proliferative components of inflammatory process. NSAIDs such as aspirin elicit a slight inhibition whereas steroidal anti-inflammation drugs have a strong inhibition on both transudative and proliferative phases of inflammation[8]. The present study showed the same effects of prednisolone and EPS extract on transudative phase. Moreover, prednisolone but not EPS extract, markedly reduced the body weight gain and the thymus weight. The results obtained suggest a difference in mechanism of anti-inflammatory activity of EPS extract and prednisolone. It is therefore postulated that EPS extract does not divide these steroidal-like activity.

Formalin test possesses two distinctive phases in which reflecting different types of pain. The early phase is due to the direct effect of formalin on nociceptors (non-inflammatory pain), whereas the late phase reflects pain from inflammation[9]. EPS extract showed anti-nociceptive effect on both phase of the formalin test, which suggests both direct effect on the nociceptor and an inhibition of inflammatory pain which in turn suggests its possible effect on the synthesis and/or release of prostaglandins and/or other pain mediators.

When injected systemically into experimental animals, exogenous pyrogens have been shown to induce the production of pro-inflammatory cytokines, such as IL-1β, IL-6, IFN-α and TNF-α, which enter the hypothalamic circulation and stimulate release of local PGs, resetting the hypothalamic thermal setpoint[16]. Antipyretic activity is commonly mentioned as one of the characteristics of aspirin and some NSAIDs. This activity is caused by their inhibitory effect on the biosynthesis and/or the release of PGE_2 into the preoptic area of the anterior hypothalamus caused by endogenous pyrogens[16,17]. In the present study, EPS extract and aspirin showed antipyretic activity causing a decrease in the body temperature of hyperthermic rats induced by brewer's yeast at all assessment times. The antipyretic activity of EPS extract might be due to the inhibition of the synthesis and/or release of local PGE_2 into hypothalamus.

Conclusion

The present study reveals the anti-inflammatory, anti-nociceptive, and antipyretic activities of EPS extract. The results obtained suggest that the mechanism of action seems to be similar to NSAIDs. Inhibition of the synthesis and/or release of inflammatory mediators may be the main mechanism (s) of action of EPS extract.

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ฤทธิ์ต้านการอักเสบระงับปวด และลดไข้ของสารสกัดเอทานอลจากรากต้นช้าพลู

สีวบูรณ์ สิรีรัฐวงศ์, สุภาพร วรรณศิริ, ศุภชัย ศรีธิวงศ์, อรุณพร อิฐรัตน์, กาญจนา ใจจ้อย

***ภูมิหลัง**: ต้นช้าพลู (Piper sarmentosum Roxb.) เป็นพืชในวงศ์ไม้พริกไทย-พลู ซึ่งทั้งต้นของช้าพลูนำมาใช้ขับเสมหะ และใบใช้เป็นยาขับลม สารสกัดหลายชนิดจากพืชในวงศ์นี้มีฤทธิ์ต้านการอักเสบ ระงับปวด และลดไข้เมื่อศึกษาในโดยใช้แบบจำลองในสัตว์ทดลอง*

***วัตถุประสงค์**: เพื่อประเมินฤทธิ์ต้านการอักเสบ ระงับปวดและลดไข้ของสารสกัดเอทานอลจากรากต้นช้าพลู*

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***ผลการศึกษา**: สารสกัดช้าพลูมีฤทธิ์ยับยั้งการบวมของใบหูหนูจากการเหนี่ยวนำด้วยเอทิล ฟีนิลโพรพิโอเลทและการบวมของอุ้งเท้าหนูขาวจากการเหนี่ยวนำด้วยคาราจีแนนได้อย่างมีนัยสำคัญทางสถิติ สารสกัดสามารถลดน้ำหนักของ ทรานซูเดทและแกรนูโลมาในแบบจำลองการอักเสบเรื้อรังโดยใช้ก้อนสำลีเหนี่ยวนำให้เกิดการสร้างแกรนูโลมาในหนูขาวได้ สารสกัดยังมีฤทธิ์ยับยั้งความเจ็บปวดในหนูถีบจักรจากการใช้ฟอร์มาลินเหนี่ยวนำทั้งในระยะแรกและ ระยะหลังของการทดลอง นอกจากนี้ยังมีฤทธิ์ลดไข้ที่เกิดจากใช้ยีสต์เหนี่ยวนำให้หนูขาว*

***สรุป**: สารสกัดช้าพลูมีฤทธิ์ต้านการอักเสบ ระงับปวดและลดไข้*