Research Article

Anti-Inflammatory, Analgesic, and Antipyretic Activities of the Ethanol Extract of Piper interruptum Opiz. and Piper chaba Linn.

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Piper interruptum Opiz. and Piper chaba Linn. are herbaceous plants in the Piperaceae family. The ethanol extract of P. interruptum and P. chaba inhibited ethyl phenylpropiolate-induced ear edema and carrageenan-induced hind paw edema in rats. Both extracts reduced transudative and granuloma weights as well as body weight gain and thymus weight of the chronic inflammatory model using cotton pellet-induced granuloma formation in rats. Moreover, both extracts exhibited analgesic activity on both early phase and late phase of formalin test in mice and also showed antipyretic activity on yeast-induced hyperthermia in rats.

1. Introduction

The Piperaceae is a huge family of plants, which has been extensively used for traditional medicine. In Thailand, P. interruptum or P. ribesoides Wall., called Sa-kan, is commonly found in the northern and northeastern parts. The stem has long been used as carminative, antiflatulent, and tonic element [1]. There are few reports on pharmacological activity of this plant. The ethanol extract of stem showed larvicidal effect [2] and the methanol extract exhibited inhibitory activity on acetylcholinesterase enzyme [3].

P. chaba or P. longum Linn. or long pepper has various Thai local names such as Di-pli, Prik-hang, Dipli-chueak. In Thai traditional systems of medicine, the dried mature unripe fruits of this plant find wide application as carminative, element tonic, antiarrheal, expectorant and oxytocic for postlabor [4]. The fruit extract of P. chaba has been shown to exhibit various activities including anti-inflammation [5–7], hepatoprotection [8], cytotoxicity and antitumor [9], chemoprevention [10], antiangiogenesis [11], immunomodulation [12], and adipogenesis [13].

The purpose of this study was to evaluate anti-inflammatory, analgesic, and antipyretic activities of ethanol extract from stem of P. interruptum and fruit of P. chaba in animal models.

2. Materials and Methods

2.1. Plant Materials. Stem of P. interruptum (PI) was collected from Phupan, Sakhonnakhon and fruit of P. chaba
(PC) from Khaosaming, Chanthaburi, Thailand. The certifi-
cations of plant materials were carried out at the herbarium
of Department of Forestry, Bangkok whereas the herbarium
vouchers have been kept at the herbarium of Southern
Center of Thai Medicinal plant at Faculty of Pharmaceutical
Science, Prince of Songkla University, Songkhla, Thailand.
SKP 146160901 and SKP 146160301 are voucher numbers of
P. interruptum and P. chaba, respectively.

2.2. Preparation of Plant Extracts. All plant materials were
cleansed using water to reduce microbial load. Next, they
were cut into small pieces and dried at 50 °C, powdered, and
dried by evaporator. The maceration was repeated twice
and then dried by evaporator. The percentage yields of PI and
PC extracts were calculated as 2.47 and 10.89, respectively.

2.3. 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 National
Laboratory Animal Center, Nakorn Pathom, Thailand. Six
animals were randomly assigned to each group of control
and treatment. They were kept in a room maintained under
environmental conditions of 25 ± 1°C and 12 h dark-light
period. The animals had free access to water and food. Rats
were kept in the 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). After the experiments, all animals were
sacrificed.

2.4. Anti-Inflammatory Study

2.4.1. Ethyl-Phenylpropiolate-(EPP-) Induced Ear Edema in
Rats [14]. Male rats weighing 40–60 g were used for topical
application of EPP 1 mg/20 μL/ear to induce edema on the
inner and outer surface of both ears. The test substances
were applied in the same manner in a volume of 20 μL prior
to applying the irritants. Control group received acetone
(20 μL/ear), and the reference group received 1 mg/20 μL/ear
of phenylbutazone in acetone. The test group received PI or
PC extract at the dose of 1 mg/20 μL/ear. The thickness of
each ear was measured with a digital vernier caliper before
and at 15, 30, 60, and 120 min after edema induction.

2.4.2. Carrageenan-Induced Paw Edema in Rats [15]. Male
rats weighing 100–120 g were divided into five groups of six
rats each. The test groups were received PI or PC extract (300,
600, 1,200 mg/kg), aspirin (300 mg/kg), and 5% Tween80
(control). The test substances were orally administered 1
hour 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 rat. The edema volumes were determined using
a plethysmometer (model 7140, Ugo Basile, Italy) before and
1, 3, and 5 h after carrageenan injection.

2.4.3. Cotton Pellet-Induced Granuloma Formation in Rats
[16]. Male rats of weight range 200–250 g were chosen
in this experiment. Two sterilized cotton pellets (20 ±
1 mg) were implanted subcutaneously; one on each side
of the abdomen under thiopental anesthesia. The PI or
PC extract (1,200 mg/kg), aspirin (300 mg/kg), and pred-
nisolone (5 mg/kg) were orally administered once daily in
a dosage regimen for 7 days, whereas the control group
received 5% Tween only. On the 8th day after implantation,
rats were anesthetized with thiopental sodium (50 mg/kg,
intraperitoneally). Both cotton pellets and thymus were
dissected, dried at 60°C for 18 h and their dry weight
determined. Change in body weight from the beginning and
the end of experiment was also recorded. The transudative
and granuloma weights were calculated as well as percent
granuloma inhibition of the test drugs.

2.5. Analgesic Study

2.5.1. Formalin Test in Mice [17]. Male ICR mice weighing
30–40 g were divided into six groups of six animals each.
Control group received 5% Tween80. Aspirin (300 mg/kg)
and morphine (10 mg/kg) were used as a reference group. PI
and PC extract were administered at the doses of 300, 600,
and 1,200 mg/kg. The formalin test consists of two distinctive
phases. For the early phase assessment, test substances were
orally administered to male ICR mice 1 h, whereas morphine
was injected intraperitoneally 30 min before the formalin
injection. Twenty μL of 1% formalin in NSS was injected
subcutaneously into the left dorsal hind paw. Time mice
spent on licking of the injected hind paw was determined
between 0–5 min after the formalin injection. In the late
phase, formalin was injected after oral administration of test
substances for 40 min, or morphine injection for 10 min.
Next, the licking time was determined between 20 and
30 min after the formalin injection.

2.6. Antipyretic Study

2.6.1. Yeast-Induced Hyperthermia in Rats [18]. Before
inducing pyrexia, the initial rectal temperature of male rats
weighing 200–250 g was recorded using a twelve-channel
electric thermometer (LETICA, model TMP 812 RS, Panlab
S.L., Spain). Next, hyperthermia was induced by subcuta-
neous injection of 1 mL/100 g body weight of 25% yeast in
NSS. The rectal temperatures were recorded 18 h later. Then,
those animals which show a rise in rectal temperature of
more than 1°C were orally administered with test substances;
PI and PC extracts were (300, 600, 1,200 mg/kg), aspirin
(300 mg/kg), and 5% Tween80 (control group). The rectal
temperature of animals was recorded every 30 min for 2 h
following the treatment.

2.7. Statistical Analysis. Results were expressed as mean ±
standard error of mean (S.E.M.). Statistical significance was
determined by one-way analysis of variance (ANOVA) and
post hoc least-significant difference (LSD) test using SPSS
software (version 11.0). The P values less than 0.05 were
considered significant.
3. Results

3.1. Anti-Inflammatory Activities of Piper interruptum Opiz. and Piper chaba Linn. The EPP-induced rat ear edema is suitably a common model for screening and estimating anti-inflammatory activity of test substances. Topical application of EPP on rat ears produced a marked edema formation as shown in Figure 1. PI and PC extract at the dose of 1 mg/ear significantly inhibited the ear edema formation. As a positive control, phenylbutazone (1 mg/ear) exhibited significant inhibitory activity on the ear edema formation at all determination times.

Next, we used the carrageenan-induced paw edema model to further confirm the activities of the extracts. As depicted in Figure 2, PI extract, at doses of 300, 600, and 1,200 mg/kg reduced the paw edema at 1, 3, and 5 h after carrageenan injection. Similarly, PC extract, at doses of 1,200 mg/kg significantly reduced the paw edema at 3 and 5 h after carrageenan injection. The positive control aspirin (300 mg/kg) markedly produced significant inhibitory effect of the paw edema at all assessment times.

The cotton pellet-induced granuloma formation in rats was further conducted to determine whether the extracts are able to inhibit the chronic inflammation. The positive control group treated with prednisolone (5 mg/kg, p.o.), daily for 7 days elicited a noticeable inhibition on transudation and granuloma formation (Table 1). In contrast, aspirin (300 mg/kg) did not reduce both parameters. PI extract at the dose of 1,200 mg/kg decreased transudative weight, whereas PC extract reduced both transudative and granuloma weights. In addition, PI and PC extracts, similar to prednisolone, significantly decreased the body weight gain and thymus weight of animals while aspirin did not affect those parameters (Table 2).

3.2. Analgesic and Antipyretic Activities of Piper interruptum Opiz. and Piper chaba Linn. The formalin test is an applicable and reliable model of nociception. As shown in Figure 3, all doses of PI and PC extracts, aspirin, and morphine elicited significant inhibitory effect on the formalin test in mice. Significantly, the PI extract had a much smaller effect in the early phase than the other treatments.

In the antipyretic study, PI extract at doses of 300, 600, 1,200 mg/kg and aspirin significantly decreased the rectal temperature of hyperthermia rats at all recorded times (Figure 4(a)). Likewise, PC extract at the doses of 600 and 1,200 mg/kg significantly reduced the rectal temperature of rats as shown in Figure 4(b).
4. Discussion

Results of the present study reveal that ethanolic extracts of *Piper interruptum* Opiz. and *Piper chaba* Linn. possesses anti-inflammatory, analgesic, and antipyretic activities in animal models.

During the acute phase of inflammation, key inflammatory mediators including histamine, serotonin, bradykinin, and prostaglandin are released in order to promote vasodilation and vascular permeability as well as edema [19]. In the present study, PI and PC extracts showed the inhibitory effect on the ear edema formation induced by EPP. This result suggested that PI and PC extracts possessed anti-inflammatory activity by blocking the inflammatory mediators of the acute phase of inflammation.

The carrageenan-induced paw edema is considered as a model of the acute phase of inflammation which is widely used for discovery and investigation of anti-inflammatory drug [15]. Especially, this model is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of NSAIDs, which involves the inhibition of PGs synthesis [20]. Paw edema is processed by several inflammatory mediator released in ordinate sequence. The initial phase is caused by the release of histamine, serotonin, and bradykinin and followed by the release of prostaglandins (PGs) at the 3rd hour and lasts for about 6 hour after carrageenan injection [20]. The final phase appears to be most interesting in which the maximal vascular response occurs with the leukocyte migration to the inflamed area [21]. In the present study, oral administration of PI or PC extract suppressed the hind paw edema. The significant inhibitory effect of both extracts on carrageenan-induced paw edema at the 3rd hour suggests that the underlying mechanism of action of PI and PC extracts may involve blocking of the PGs synthesis and/or the other inflammatory mediators.

Cotton pellet-induced granulation is commonly used to assess the transudative and proliferative components of chronic inflammation. The subcutaneous implantation of cotton pellet in rat has been divided into at least three response phases, transudative phase, exudative phase, and proliferative phase. The transudative phase is defined as the increase in wet weight of the pellet that occurs during first 3 hour. An exudative phase is defined as plasma leaking from bloodstream around the granuloma that occurs between 3 and 72 hour after the implantation of pellet. The proliferative phase is measured as the increase in dry weight of the granuloma that occurs between 3 and 6 days after the implantation [16]. In addition, the implanted material induces a host inflammatory response and modulates the release of inflammatory mediators which finally lead to tissue proliferation and granular formation [22–24]. NSAIDs such as aspirin elicit a slight inhibition, whereas steroidal anti-inflammatory drugs strongly inhibit both transudative and proliferative phase of inflammation [16]. In this study, similar to prednisolone, PI and PC extract showed similar effects on both transudative and proliferative phases and reduced the body weight gain as well as the thymus weight. The results obtained suggest a mechanism of anti-inflammatory activity of PI and PC extract as steroidal-like effects.

The formalin test elucidates central and peripheral activities of nociception. The response pattern has two distinct periods of intensive licking activity, an early response (0–5 min after injection) and a late response (20–30 min after injection). The early phase is due to direct effect of formalin.
on nociceptors (noninflammatory pain). The late phase response is development of an inflammatory response and release of analgesic mediators, which reflect inflammatory pain [17]. Experimental results have indicated that substance P and bradykinin participate in the early phase, whereas histamine, serotonin, bradykinin, and PGs are involved in the late phase [25]. Our study showed analgesic activity of PI and PC extracts on both phases of the formalin test suggesting both direct analgesic effects on the nociceptor and an inhibition of inflammatory pain. Thus, their mode of action possibly involves the synthesis and/or release of PGs and/or other pain mediators.

Fever is provoked by many exogenous substances in animal models, including bacterial endotoxins and microbe infection. Exogenous pyrogen induces the production of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), IL-6, interferon-α (IFN-α), and tumor necrosis factor-α (TNF-α), which enter hypothalamic circulation and stimulate the release of local prostaglandins (PGs), thereby resetting the hypothalamic thermal setpoint [26]. Like aspirin, both PI and PC extract showed antipyretic activity which is likely due to inhibition of the synthesis and/or release of local PGE₂ into the preoptic area of anterior hypothalamus [26, 27].

5. Conclusion

The overall results demonstrate that extracts of *Piper interruptum* Opiz. and *Piper chaba* Linn. have anti-inflammatory, analgesic, and antipyretic activities in laboratory animals. The results obtained suggest their mechanism of action similar to NSAIDs as well as steroid-like effect. Nonetheless, the precise mechanism and the bioactive principles responsible for these actions remain to be elucidated.

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References


