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Research Paper

Investigation of anti-inflammatory, antinociceptive and antipyretic activities of *Stahlianthus involucratus* rhizome ethanol extract

Phornchai Pingsusaen [a], Puongtip Kunanusorn [a,*], Parirat Khonsung [a], Natthakarn Chiranthanut [a], Ampai Panthong [a], Chaiyong Rujjanawate [b]

[a] Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
[b] School of Medicine, Mae Fah Luang University, Chiang Rai 57100, Thailand

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ABSTRACT

Ethnopharmacological relevance: *Stahlianthus involucratus* (Zingiberaceae) has long been used in traditional medicine to treat inflammation, pain, and fever. However, no pharmacological study of this plant has been reported to confirm these activities. The aim of this study was to investigate the anti-inflammatory, antinociceptive and antipyretic activities of *Stahlianthus involucratus* rhizome ethanol extract (SiE) in animal models.

Materials and methods: Anti-inflammatory activity of SiE was investigated in rats using ethyl phenylpropiolate (EPP)-induced ear edema, carrageenan- and arachidonic acid (AA)-induced hind paw edema, and cotton pellet-induced granuloma formation models. Acetic acid-induced writhing response in mice and tail-flick test in rats as well as yeast-induced hyperthermia in rats were used to investigate the antinociceptive and antipyretic activities, respectively.

Results: SiE significantly inhibited EPP-induced ear edema, carrageenan- and AA-induced hind paw edema. Its inhibitory effect in carrageenan-induced hind paw edema seemed to be in a dose-dependent manner. In cotton pellet-induced granuloma formation, SiE showed suppressive effects on granuloma formation but not on body weight gain and dry thymus weight. It could normalize serum alkaline phosphatase activity to nearly normal level. SiE also possessed a significant inhibitory effect, which seemed to be dose-dependent, on acetic acid-induced writhing response, whereas only at the highest dose of SiE could significantly increase test reaction time at all time-points in tail-flick test. However, no antipyretic activity was observed.

Conclusions: These results suggest that SiE possesses anti-inflammatory and antinociceptive, but not antipyretic, activities. This study therefore rationalizes the traditional use of SiE for the treatment of inflammation and pain.

1. Introduction

Stahlianthus involucratus (King) Craib ex Loes, a perennial herbaceous plant of the Zingiberaceae family, is widely distributed in the forest and on the mountains of many countries in Asia, including Thailand (Chaveerach et al., 2007). Its local names are “Wan Phet Yai” in Thai, “Tu Tian Qi” in Chinese and “Easkine” in Bangladesh. In Bangladesh folk medicine, the plant has been claimed to treat inflammatory disorders and fever (Yusuf et al., 2007). Traditional healers in Thailand also topically apply the crushed rhizome to relieve muscle pain and edema.

Several pharmacological activities of plants in Zingiberaceae family including anti-inflammatory, analgesic, and antipyretic activities have been shown in many scientific reports (Somchit et al., 2005; Thenmozhi et al., 2013; Azam et al., 2014). The most recognized anti-inflammatory effect of rhizomes of several Zingiberaceae species has consistently reported and confirmed. The hexane extract as well as the active constituents of *Curcuma xanthorrhiza* Roxb. has been shown to inhibit carrageenan-induced paw edema in rats (Claeson et al., 1993). The rhizomes of *Zingiber officinale* and *Alpinia officinarum* contain potent inhibitors against prostaglandin (PG) synthetase, an enzyme of PG biosynthesis and arachidonate 5-lipoxygenase, an enzyme of leukotriene (LT) biosynthesis (Kiuchi et al., 1992). The hydroalcoholic extract of *Zingiber officinale* rhizomes also reduces carrageenan-induced paw swelling in rats (Penna et al., 2003).

Abbreviations: SiE, *Stahlianthus involucratus* rhizome ethanol extract; EPP, ethyl phenylpropiolate; AA, arachidonic acid; PG, prostaglandin; LT, leukotriene; GC/MS, gas chromatography/mass spectrometry; DMSO, dimethylsulfoxide; NSS, normal saline solution; AP, alkaline phosphatase; MPE, maximum possible effect; COX, cyclooxygenase; NSAIDs, non-steroidal anti-inflammatory drugs

* Corresponding author. Tel.: +66 53 945353; fax: +66 53 945355.
E-mail address: puongtip.k@cmu.ac.th (P. Kunanusorn).

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Mukophadhyay et al. (1982) demonstrated the activity of curcumin, an active constituent of *Curcuma longa* L. against carrageenan-induced rat paw edema and cotton pellet granuloma models of inflammation in rats. The biologically active compounds of Zingiberaceae plants that exert anti-inflammatory activity include, for example, gingerol and shogaol of *Zingiber officinale* Roscoe extract (Dugasani et al., 2010); diarylheptanoid of *Alpinia officinarum* extract (Yadav et al., 2003; Yasukawa et al., 2008), and curcuminoids especially curcumin of *Curcuma longa* L. extract (Huang et al., 1991; Jobin et al., 1999).

Despite those traditional claims of *Stahlianthus involucratus*, the scientific evidence for its pharmacological as well as phytochemical properties has not been reported yet. The present study aimed to investigate the anti-inflammatory, antinociceptive and antipyretic activities of the ethanol extract from *Stahlianthus involucratus* rhizomes in animal models.

2. Materials and methods

2.1. Plant material and extraction

The rhizomes of *Stahlianthus involucratus* were collected in April 2009 from Chiang Rai Province, Thailand. The plant was authenticated by one of the authors (Dr. Rujjanawate) and the voucher specimen (no. 144) has been deposited at the School of Medicine, Mae Fah Luang University, Chiang Rai, Thailand. The air dried (at room temperature) rhizome powder was macerated with 95% ethanol for 2 d and filtered. The marc was remacerated two times in the same manner as the initial maceration and filtered. The combined filtrate was concentrated in vacuo at 55 °C and lyophilized to obtain a dry ethanol extract (SiE, 6% w/w yield).

2.2. Gas chromatography/mass spectrometry (GC/MS) analysis

GC/MS analysis of SiE was carried out on a gas chromatograph (GC 7890 Agilent Technologies) fitted with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The GC oven temperature was programmed at 50 °C, held for 5 min, raised to 200 °C at 10 °C/min, then to 250 °C at 5 °C/min and held for 10 min. The injection temperature was 250 °C; the flow rate of carrier gas, helium, was at 1.5 mL/min; 1:25 split ratio. The gas chromatograph was coupled to a mass selective detector (Agilent HP 5973). The MS operating parameters were as follows: ionization voltage, 70 eV; ion source temperature, 230 °C. Identification of the extract components was performed by comparison of their relative retention times and mass spectra with those in the NIST05a.L Database (Agilent Technologies Inc.).

2.3. Experimental animals

Male Sprague–Dawley rats and male Swiss albino mice were purchased from the National Laboratory Animal Center, Mahidol University, Nakorn Pathom, Thailand. All animals were acclimatized for at least 1 week in a room maintained under environmentally controlled conditions of 23 ± 2 °C and a 12 h light–dark cycle before starting the experiments. They had free access to water and standard diet (Pokphan Animal Feed Co. Ltd., Bangkok, Thailand), but only the diet was withdrawn 12 h before dosing. All experimental procedures were approved by the Animal Ethics Committee of the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand (Protocol Number 8/2553).

2.4. Drugs and chemicals

Diclofenac sodium, lambda carrageenan and arachidonic acid (AA) were purchased from Sigma Chemical Company (St. Louis, U.S.A.). Ethyl phenylpropiolate (EPP) and Brewer's yeast were purchased from Fluka Chemicals Co. Ltd. (Japan). Codeine phosphate and acetic acid were purchased from the Government Pharmaceutical Organization (Thailand). Prednisolone was purchased from Schering, Bangkok Ltd. (Thailand). All other chemicals were of analytical grade.

2.5. Test substance administration

SiE and reference drugs were orally administered in an equivalent volume of 0.2 mL/100 g body weight of rats and 0.1 mL/10 g body weight of mice except those rats in the EPP-induced ear edema model of which the test substances were applied topically (20 μL/ear). Tween 80 (5%) was used as vehicle for SiE in all experimental models except in the ear edema model in which acetone and 5% dimethylsulfoxide (DMSO) in acetone were used as vehicles for the extract and diclofenac, respectively. In the control group, animals received only the vehicle in the same volume and the same route.

2.6. Anti-inflammatory activity

2.6.1. EPP-induced ear edema

The method described by Brattsand et al. (1982) was used with slight modification, to assess topical anti-inflammatory activity of SiE. Male rats of 40–60 g body weight were randomly divided into four groups of three rats each. EPP at the dose of 1 mg/ear was dissolved in acetone and topically applied to the inner and outer surfaces of both ears. The vehicle, SiE (5 mg/ear) or diclofenac (5 mg/ear), was applied to the ear just before EPP application. The thickness of each ear was measured with the digital vernier calipers before and at 15, 30, 60 and 120 min after edema induction. The increase in ear thickness of each test group was compared with its corresponding control group and percent inhibition was calculated.

2.6.2. Carrageenan-induced hind paw edema

This method was performed as described by Winter et al. (1962). Male rats of 100–120 g body weight were randomly divided into five groups of six rats each. Carrageenan [(0.05 mL, 1%, w/v in normal saline solution (NSS)] was injected intradermally into the plantar side of the right hind paw 1 h after the administration of the vehicle, diclofenac (10 mg/kg) or SiE (75, 150 and 300 mg/kg). Paw volumes were measured using a plethysmometer (model 7150, Ugo Basile, Italy) before and at 1, 3, and 5 h after carrageenan injection.

2.6.3. AA-induced hind paw edema

Male rats of 100–120 g body weight were randomly divided into six groups of six rats each. AA [0.1 mL, 0.5%, w/v in 0.2 M carbonate buffer (pH 8.4)] was injected intradermally into the plantar side of the right hind paw 2 h after the administration of the vehicle, diclofenac (10 mg/kg), prednisolone (5 mg/kg), or SiE (75, 150 and 300 mg/kg). Paw volumes were measured using a plethysmometer (model 7150, Ugo Basile, Italy) before and at 1 h after AA injection (Di Martino et al., 1987).

2.6.4. Cotton pellet-induced granuloma formation

The method was slightly modified from that of Swingle and Shideman (1972). Male rats weighing 180–200 g were randomly divided into five groups of six rats each. Two sterilized cotton pellets (20 ± 1 mg) were implanted subcutaneously, one on each side of the abdomen in all groups except in the normal group, under light ether anesthesia. Rats in groups I (normal group) and II (control group) received 5% Tween 80. Rats in groups III and IV received diclofenac and prednisolone, respectively, at the same