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Antinociceptive activity of the methanolic extract of *Kaempferia galanga* Linn. in experimental animals

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ABSTRACT

Kaempferia galanga Linn. (Zingiberaceae) presents many chemical constituents of the volatile oil extracted from the rhizome. The rhizome of *Kaempferia galanga* is used by people in many regions for relieving toothache, abdominal pain, muscular swelling and rheumatism. In this study we investigated the antinociceptive activity in mice and rats using acetic acid-induced writhing, formalin, hot plate and tail-flick tests. The extract at test doses of 50, 100 and 200 mg/kg, p.o. clearly demonstrated antinociceptive activity in all tests. This activity was dose- and time-dependent. The extract administered at 200 mg/kg, p.o. had a stronger antinociceptive effect than aspirin (100 mg/kg, p.o.) but less than morphine (5 mg/kg, s.c.). Naloxone (2 mg/kg, i.p.) abolished the antinociceptive action of both morphine (5 mg/kg, s.c.) and the extract (200 mg/kg, p.o.) in a similar manner. In conclusion, the methanol extract of *Kaempferia galanga* markedly demonstrated the antinociceptive action in experimental animals. The antinociceptive mechanisms appear to be both peripherally and centrally mediated actions and the opioid receptors are probably involved. Therefore, our studies support the use in traditional medicine of *Kaempferia galanga* against pain caused by various disorders.

1. Introduction

Medicinal plants are important sources of new chemical substances that potentially have strong therapeutic effects. Most people living in developing countries are almost completely dependent on traditional medical practices for their primary health care needs and higher plants are known to be the main source for drug therapy in traditional medicine (Calixto, 2005). *Kaempferia galanga* Linn. (Zingiberaceae), commonly called "Proh Hom" in Thai, is an acaulescent perennial growing in Southern China, Indochina, Malaysia, India and Thailand. This medicinal plant has been extensively used for treatment of various disorders including hypertension, rheumatism and asthma (Zakaria and Mustafa, 1994). In Thailand, the rhizome of *Kaempferia galanga* is used by people in many regions for relieving toothache, abdominal pain, muscular swelling and rheumatism (Sirirugsa, 1997). In addition, the ethnomedical uses of aerial part of *Kaempferia galanga* are claimed in Thai traditional text books; the stem is used for menstrual stimulation and in the treatment of dyspepsia, whereas leave and flower are used for the treatment of *Tinea versicolor*, and eye diseases and seizures, respectively (Pongboonrod, 1976; Thamaree and Tankeyoon, 1981; Sighabutra, 1993). The most common indications for its use, besides hypertension, include rheumatism, asthma, headaches, cough, toothaches and as a poultice for applying to bruises and wounds (Perry and Metzger, 1980). In Malaysia and Indonesia, this plant is used to make a gargle, the leaves and rhizomes are chewed to treat coughs, or pounded and used in poultices or lotions applied to relieve many ailments; the juice of the rhizome is used as an expectorant and carminative, and is often a part of children's medicine and tonics (Othman et al., 2006). The rhizome is also used to treat abdominal pain, and as an embrocation or sudorific to treat swelling and muscular rheumatism (Othman et al., 2006). In China, this plant is used as a remedy for toothache, as a stimulant, carminative to treat cholera, and to treat contusions, chest pains, headache and constipation (Ibrahim and Rahman, 1988; Mustafa et al., 1996). Additionally, the rhizomes of the plant that contains essential oils have been used in a decoction or powder for indigestion, cold, pectoral and abdominal pains, headache and toothache (Kanjanapothi et al., 2004). Its alcoholic maceration has also been applied as a liniment for rheumatism (Keys, 1976; Lieu, 1990). Some pharmacological activities of *Kaempferia galanga* have been reported, for example, as a smooth muscle relaxant and vasorelaxant (Mustafa et al., 1996; Othman et al., 2006).

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Kaempferia galanga possesses several bioactivities and is widely used in Thai and other Asian traditional medicine but so far its antinociceptive activity has not been investigated. Phytochemical studies carried out with *Kaempferia galanga* revealed many different volatile oils. The major chemical constituents of the volatile oil extracted from dried rhizome were ethyl-*p*-methoxycinnamate (31.77%), methylcinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%) and pentadecane (6.41%), respectively (Tewtrakul et al., 2005). Other constituents of the rhizome include cineol, borneol, 3-carene, camphene, kaempferal, cinnamaldehyde, *p*-methoxycinnamic acid, ethyl cinnamate and ethyl *p*-methoxycinnamate (Nakao and Shibu, 1924). A methanolic extract of the rhizome contains ethyl *p*-methoxy-*trans*-cinnamate, which is highly cytotoxic to HeLa cells (Kosuge et al., 1985).

In the present study, *Kaempferia galanga* was selected because it is one of the medicinal plants commonly used in remedies to treat abdominal pain, toothache, swelling and rheumatism in Thai traditional medicine and other countries in Southeast Asia. However, up to date no ethnopharmacological data have previously been systematically conducted to evaluate the antinociceptive action supporting traditional uses of this plant in folklore medicine. In this work we evaluate the "Proh Hom" antinociceptive activity in experimental animals using methanol extract. The reason to use methanol extract in this investigation is that methanol is more non-polar than water, therefore, several substances including volatile oils, the major chemical constituents of *Kaempferia galanga*, would be expected to be more soluble in methanol fraction than in water extract.

2. Material and methods

2.1. Plant material

Kaempferia galanga were collected in January 2006 from the Chana District, Songkhla Province, Thailand. The plant was identified by Prof. Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University. A voucher specimen no. 200601 of the plant material has been deposited in the Prince of Songkla University Herbarium, Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

2.2. Experimental animals

Male Swiss albino mice and Wistar rats were used in the experiments. All animals obtained from the Southern Laboratory Animal Facility, Prince of Songkla University, Hat Yai, Songkhla, Thailand, were kept in a room maintained under standard environmentally controlled conditions of $24 \pm 1\,^{\circ}C$ and 12 h light–12 h dark cycle. They were supplied *ad libitum* with standard rodent diets and water. All experiments were approved by Animal Ethics Committees, Prince of Songkla University, Thailand.

2.3. Preparation of the plant extract and reference drugs

20 kg of the fresh plant *Kaempferia galanga*, including rhizomes and aerial parts (stems and leaves), was cleaned with tap water and air-dried at room temperature (28–30 °C). The dried plant material was pulverized by an electric blender to give 5 kg of a coarse powder. The powder obtained was macerated with 10 l of methanol and left for 7 days at room temperature. The maceration process was repeated twice and the combined extracts were filtered by suction through a Buchner funnel fitted with a Whatman No. 40 filter paper. The filtrate was evaporated at 40–60 °C under reduced pressure by a rotary evaporator and lyophilized by freeze-drying to give a total semi-solid residue of 119.2 g (yield 0.6 %, w/w) which was stored in a closed bottle and kept in a refrigerator at temperature below 4 °C until tested. The methanolic extract of *Kaempferia galanga* (MEKG) at doses of 50, 100 and 200 mg/kg was prepared by suspending this residue in the cosolvent (propylene glycol:Tween 80:distilled water; 4:1:5). This solution and cosolvent controls were given to the animals orally. The reference drugs used in this study were aspirin (100 mg/kg), morphine sulphate (5 mg/kg) and naloxone (2 mg/kg). All of reference drugs were prepared by dissolving in 0.9% normal saline.

2.4. Assessment of antinociceptive activity of MEKG

2.4.1. Acetic acid-induced writhings in mice

The method previously described was used to evaluate the antinociceptive activity (Koster et al., 1959). The extract at doses of 50, 100 or 200 mg/kg was administered orally to each mouse 30 min before intraperitoneal injection with 0.6% acetic acid in 0.9% normal saline (10 ml/kg body weight) to induce the characteristic writhings. Cosolvent (10 ml/kg, p.o.) and aspirin (100 mg/kg, p.o.) were given to mice in the control and reference groups, respectively. The mice were observed and counted for the number of abdominal constrictions and stretchings in a period of 0–20 min. The responses in the treated groups were compared with those of animals in the control group. The percentage of inhibition of the number of writhings was calculated.

2.4.2. Formalin test in mice

The method was used according to previously described (Hunskaar et al., 1985). The control group received cosolvent (10 ml/kg, p.o.) and reference groups received aspirin (100 mg/kg, p.o.) and morphine sulphate (5 mg/kg, s.c.). MEKG at doses of 50, 100 and 200 mg/kg were administered orally to the animals. After 30 min of treatment (except only 15 min for morphine), 20 μl of 2.5% formalin in saline was injected subcutaneously into a hind paw of each mouse. The time spent in licking the injected paw was recorded and expressed as the total licking time in early phase (0–5 min) and late phase (15–30 min) after formalin injection.

2.4.3. Hot plate test in mice

The hot plate test was used as previously described (Woolfe and MacDonald, 1944). The animals in the control group received cosolvent (10 ml/kg, p.o.) while the reference groups were treated with morphine sulphate (5 mg/kg, s.c.) and naloxone (2 mg/kg, i.p.). The animals in the test groups were treated with different doses (50, 100 and 200 mg/kg, p.o.) of *Kaempferia galanga*. In the remaining groups, the animals received naloxone (2 mg/kg, i.p.) 10 min before morphine (5 mg/kg, s.c.) or the extract (200 mg/kg, p.o.). After 30 min of treatment with all test drugs (except only 15 min for morphine and 10 min for naloxone), mice were placed on a hot plate maintained at $55 \pm 1\,^{\circ}C$. The latency of nociceptive response (reaction time) of each mouse that was identified by the time for licking and flicking of a hind limb or jumping was recorded. The reaction time was measured every 15 min in a 90-min period at intervals of 30, 45, 60 and 90 min. The cut-off time of observation was 45 s. Only mice that showed a nociceptive responses within 15 s was used in the experiments.

2.4.4. Tail-flick test in rats

The experiment was carried out by the method previously described (D'Amour and Smith, 1941). The experiment was carried out in the same manner as the hot plate test. After 30 min of treatment with all test drugs (except only 15 min for morphine and 10 min for naloxone), the tail-flick response of the rat was measured by gently placing the rat tail at a central position of a light beam. The time taken by the animals to withdraw (flick) its tail from heat