

## Evaluation of antinociceptive activity of the ethanolic extract from *Scaphium lychnophorum* (Hance) Pierre fruit in mice

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### Abstract

The antinociceptive activity of the ethanolic extract from the fruit of *Scaphium lychnophorum* was assessed in mice using *in vivo* animal models included the acetic acid-induced writhings, formalin, and hot plate tests. The acute toxicity of the ethanolic extract was also performed in mice. The results demonstrated that the ethanolic extract from the fruit of *Scaphium lychnophorum* markedly showed the antinociceptive activity at doses of 50, 100 and 200 mg/kg, po when compared with the control ( $p < 0.05$ ). The estimated LD<sub>50</sub> in mice was more than 5 g/kg.

**Keywords:** *Scaphium lychnophorum*, Antinociceptive, Ethanolic extract

### Introduction

*Scaphium lychnophorum* (Hance) Pierre is in Sterculiaceae family which is commonly known in Thai as Samrong. This plant is mainly distributed in Vietnam, Thailand (Ubon Ratchathani and Chanthaburi), Malaysia, Indonesia, as well as South China (1). It has been used in folk medicine for relieving various symptoms such as pain, cough and clear phlegm, and used as antipyretics. This study examined the antinociceptive potential of ethanol extract in mice using different concentrations (50, 100 and 200 mg/kg). The aim of this study was to investigate the antinociceptive activity from fruits of *Scaphium lychnophorum* (Hance) Pierre in animal models.

### Materials and methods

**Plant material:** The plant collected from Chanthaburi province which is located in the East of Thailand.

**Experimental animals:** Male Swiss albino mice were used in the experiments. All animal obtained from the southern Laboratory Animal Facility, Prince of Songkla University, Hat Yai, Songkhla, Thailand, were kept in room of controlled conditions of  $24 \pm 1^\circ\text{C}$  and 12 h light – 12 h dark cycles. All experiments were approved by Animal Ethics Committees, Prince of Songkla University, Thailand.

**Preparation of the plant extract and reference drugs:** 2.6 kg of the dried fruit *Scaphium lychnophorum* (Hance) Pierre was pulverized to give 2.6 kg of coarse powder. The powder obtained was macerated 2 times with 7.5 L and 5.5 L of ethanol, respectively, and left for 7 days at room temperature. The combined filtrate was filtered, and the filtrate was evaporated under reduced pressure and lyophilized to give a total semi-solid brownish-green residue of 41.57 g (yield 1.6%, w/w) which was stored and kept in temperature below  $4^\circ\text{C}$  until tested. The ethanolic extract of *Scaphium lychnophorum* (Hance) Pierre (EESL) at doses of 50, 100

and 200 mg/kg were prepared in cosolvent (distilled water: Tween 80: propylene glycol; 5:1:4). Aspirin, morphine sulphate and naloxone were used as reference drugs.

### Assessment of antinociceptive activity

**Acetic acid-induced writhings:** 0.6% acetic acid in 0.9% normal saline was intraperitoneally injected in mice (10 ml/kg). The EESL at doses of 50, 100 and 200 mg/kg were given orally to the test groups. Cosolvent (10 ml/kg, po) and aspirin (200 mg/kg, po) were given to mice in the control group. The mice were observed and counted for the number of abdominal constrictions and stretchings in a period of 0-20 min. This method was done as previous described (2).

**Formalin test:** The control and reference groups received cosolvent (10 ml/kg, po) and aspirin (200 mg/kg, po), respectively. The EESL at doses of 50, 100 and 200 mg/kg were given orally to the test groups. After 30 min of treatment (except only 15 min for morphine), 20 $\mu$ l of 2.5% formalin in saline was injected subcutaneously into hind paw of each mouse. The times spent in the licking hind paw in early phase (0-5 min) and late phase (15-30 min) were recorded. This method was done as previous described (3).

**Hot plate test:** The animals were placed on a hot plate at temperature of  $55 \pm 0.5$  °C of maximum time of 45 sec. The animal test groups were treated with different doses (50, 100 and 200 mg/kg, po) of the extract. Reaction times were recorded when the animals licked and flicked of hind paw or jumping at 30, 45, 60, 75 and 90 min after oral administration of the extract. This method was done as previous described (4).

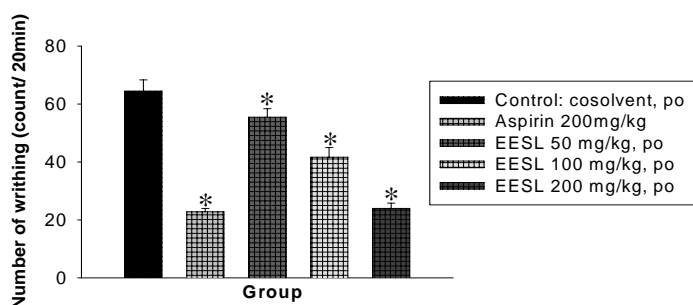
**Evaluation of acute toxicity:** The up and down procedure for acute toxicity (LD<sub>50</sub>) testing was carried out as previously described (5). Using strategy for acute toxicity testing, the animal is dosed one at a time. If an animal survives, the dose of next animal is increased. But if it died, the dose is decreased. Behavior parameters observed after administration were convulsion, hyperactivity, sedation, grooming and loss of righting reflex. Food and water were provided *ad libitum*.

**Statistical analysis:** The data obtained were analysed as a mean  $\pm$  SEM. Statistically significant differences between groups were calculated by the application of analysis of variance (ANOVA). *p*-Values less than 0.05 ( $p < 0.05$ ) were used as the significance level.

## Results

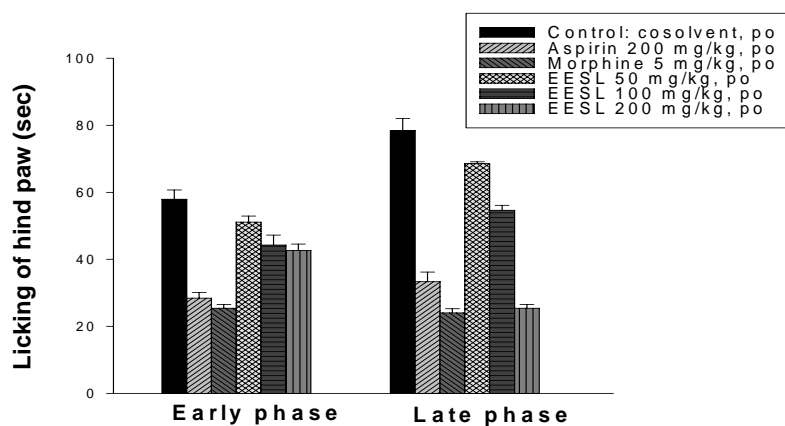
### Antinociceptive activity

**1.1 Acetic acid-induced writhings:** In acetic acid induced writhing response test, aspirin at dose 200 mg/kg and EESL at all doses used in this experiment significantly inhibited writhing compared to the control ( $p < 0.05$ ) (Fig.1). The percentage of inhibition treated with aspirin (200 mg/kg, po) was 64.6%, and with EESL at the concentrations of 50, 100 and 200 mg/kg were 13.9, 35.4 and 62.79%, respectively.



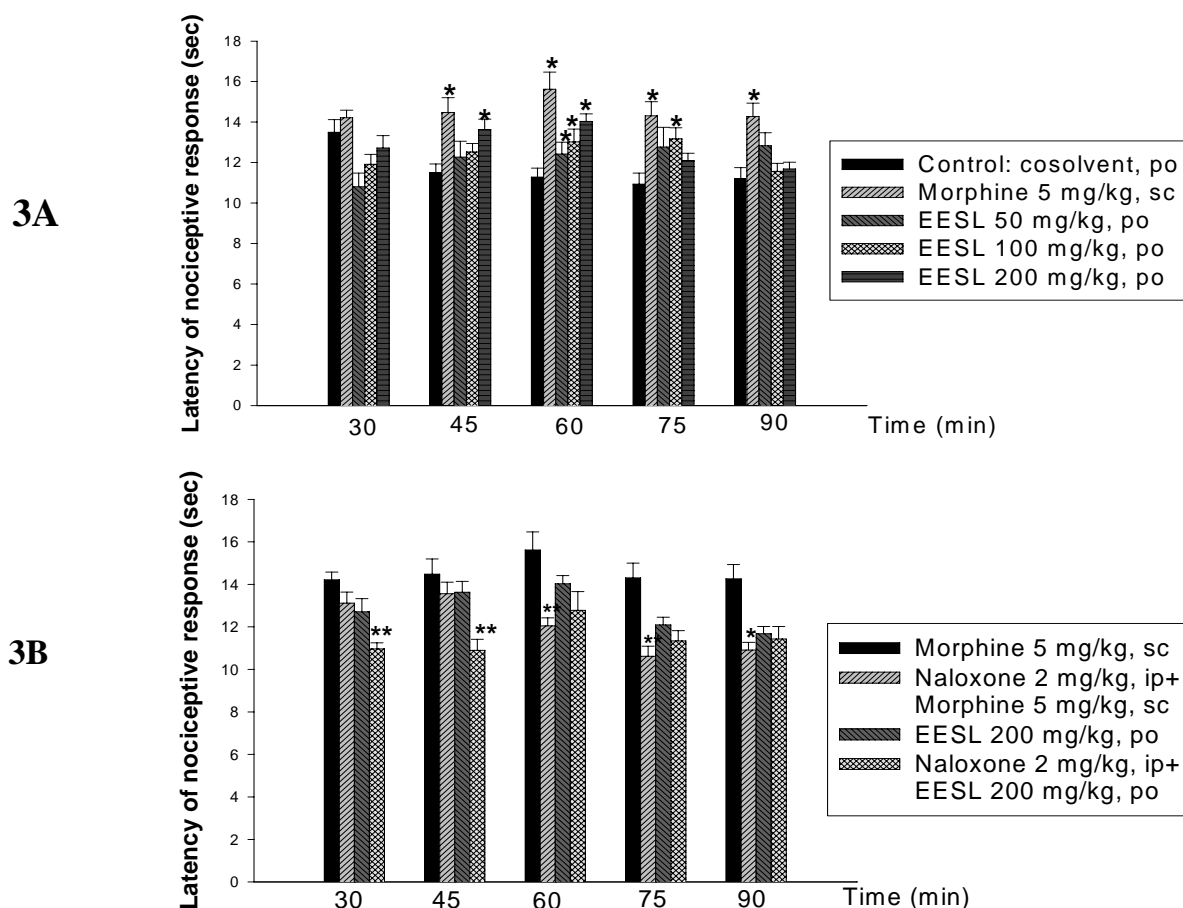
**Figure 1:** The antinociceptive activity of *S. lychnophorum* on acetic acid-induced writhing responses in mice. Each value presents mean  $\pm$  S.E.M (n=6); \* $p < 0.05$

**1.2 Formalin test:** EESL dose-dependently decreased the licking activity in early phase and late phase. In early phase, all doses of the extract, 50, 100 and 200 mg/kg significantly inhibited by 10.69, 23.5 and 26.43% respectively, and late phase they significantly inhibited by 12.61, 30.37 and 67.62%, respectively.



**Figure 2:** The antinociceptive activity of *S. lychnophorum* on formalin-induced paw licking in mice. Each value presents mean  $\pm$  SEM (n=6); \* $p$ <0.05

**1.3 Hot plate test:** EESL (50, 100 and 200 mg/kg, po) significantly exerted protective effect on heat-induced pain in mice. Naloxone (2 mg/kg, ip) before EESL (200 mg/kg, po) significantly decreased latency of nociceptive response (\*\* $p$ <0.01) (Fig. 3B).



**Figure 3:** **3A:** The antinociceptive activity of *S. lychnophorum* on heat-induced pain in mice. Each value presents mean  $\pm$  SEM (n=6); \* $p$ <0.05. **3B:** Antagonist effects of naloxone (2 mg/kg, ip.) on morphine (5 mg/kg, sc.) and ethanol extract of *Scaphium lychnophorum* (EESL) at dose 200 mg/kg, po on heat-induced pain in mice. Each value presents mean  $\pm$  SEM (n=6); \* $p$ <0.05, \*\* $p$ <0.01

**Acute toxicity:** EESL at the dose of 5g/kg, po given to mice had no affect on their behavioral responses and did not cause the mortality in mice during the observation period of 8 h and 7 days after administration. Estimated LD<sub>50</sub> in mice was more than 5 g/kg.

### Discussion and conclusion

The hot plate and formalin tests have been extensively used for evaluation of centrally acting analgesic activity. The mediator such as substance P involved in this mechanism and it acts as a neurotransmitter released from C fibers found within nociceptive primary afferent neurons into the spinal cord and mediates a part of the excitatory synaptic input to nociceptive neurons at this level (6). The writhing test generally used for screening of antinociceptive activity of various drugs. In acetic acid induced abdominal writhing test is the visceral pain model involved in the process of release arachidonic acid via cyclooxygenase and prostaglandin biosynthesis which play an important role in nociceptive mechanisms (7). In the present study, The extract at tested doses were shown to possess the antinociceptive activity in writhing and formalin and hot plate tests in dose-dependent manners. In conclusion, The EESL markedly possesses antinociceptive activity which supports the traditional uses of *S. lychnophorum* for the treatment of pain in folk medicine preparations.

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