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Analgesic activity of the hexane extract fraction from the *Abroma augusta* Linn. seed in mice

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Abstract

The effects of the n-hexane extract fraction from the seeds of *Abroma augusta* Linn. (Sterculiaceae) were assessed for its analgesic activity in mice using acetic acid-induced writhing, formalin and hot plate tests as the tested models. The extract at doses of 100, 200 and 400 mg/kg orally administered in all tests showed markedly significant antinociceptive activity when compared to control or standard drug groups.

Keywords: *Abroma augusta* Linn., Analgesic, Hexane extract fraction

Introduction

Abroma augusta Linn.(Sterculiaceae), commonly known as devils cotton, is a small tree growing wild in India, China and Thailand. The aerial parts of *Abroma augusta* has been used in traditional medicine and reported that it is used to treat joint pain, asthma, sinusitis, tonsillitis, back pain, diarrhea, influenza, and to be as antifungal and antibacterial (1). Chemical constituents that have been identified from seeds extracted with n-hexane include fatty acid composition (2). From the point of view of its traditional medicine uses, the *Abroma augusta* seeds extract (AALE) may posses an analgesic activity. Thus this study was done to evaluate the analgesic effect of the n-hexane extract fraction of *Abroma augusta* seeds in mice.

Materials and methods

Plant material and extraction: The seeds of *Abroma augusta* were used in the study. The seeds (3 kg) were air-dried at room temperature, and pulverized to powder. The powder was macerated with 6 L of n-hexane for 7 days at room temperature. The extraction process was repeated 2 times and the combined extracts were filtrated. The solvent was then evaporated under reduced pressure to give oil-like brownish extract (yield 23.02% w/w). The obtained extract was stored in a closed bottle and kept in a refrigerator at temperature below 4 °C until use.

Chemicals and Drugs: 0.6% acetic acid, aspirin, 2.5% formalin, morphine, naloxone and n-hexane were used in this study.

Animals: Male Swiss albino mice (30-40 g), obtained from the Southern Laboratory Animal Facility, Prince of Songkla University, Hat Yai, Songkhla, Thailand, were used and kept in a room maintained under the condition of 24±1 °C and 12 h light-12 h dark cycles. All animals had free access to water and standard diets. The animal Ethics Committees, Prince of Songkla University approved all experimental protocols.

Assessment of analgesic effect:

Writhing test*:* The experiment was done according to the previous described method (3). Male mice were divided into five groups of six animals. The standard drug aspirin and AALE at doses of 200 mg/kg, and 100, 200 and 400 mg/kg, respectively were orally administered to each mouse 30 min before intraperitoneal injection with 0.6% acetic acid (10 ml/kg). The number of writhes was counted for 0-20 min.

Formalin test*:* The method was done according to a previous described (4). Male mice were divided into six groups of six animals. Standard drugs (aspirin 200 mg/kg, po and morphine 5 mg/kg, sc), and AALE at doses of 100, 200 and 400 mg/kg were orally administered to each mouse. After 30 min of treatment (except only 15 min for morphine), 20 µl of 2.5% formalin was subcutaneously injected into a hind paw of each mouse. The total licking time in early phase (0-5 min) and late phase (15-30 min) after formalin injection were recorded.

Hot plate test*:* The method was used as previous described (5). Male mice were divided into eight groups of six animals. Standard drugs (morphine 5 mg/kg, sc and naloxone 2 mg/kg, ip), and AALE at doses of 100, 200 and 400 mg/kg were orally administered to each mouse. After 30 min of treatment (except only 15 min for morphine and 10 min for naloxone), mice were placed on a hot plate maintained at 55±1 °C. The latency of nociceptive responses such as licking of the hind limb or jumping were recorded at 30, 45, 60, 75 and 90 min after administration.

Evaluation of acute toxicity of the AALE : The method was done according to as previous described (6). In this study procedure, the animal received a single dose of the extract. If an animal died, the dose for the next is decreased while if it survives, the dose for the next is increased. Each animal is then observed for 2 day before dosing the next animal. The first dose was begun at 300 mg/kg to 5 g/kg adjusted by a constant multiplicative factor of 1.5 up. Behavior parameters were observed such as convulsion, hyperactivity, sedation, loss of righting reflex and increased or decreased respiration during a period of 8 h and 7 day. Food and water were given *ad libitum*.

Statistical analysis : The data are expressed as mean±S.E.M and analyzed statistically by one-way ANOVA followed by LSD test. $P<0.05$ was considered significant in all cases.

Results

Writhing test

The AALE at doses of 100, 200 and 400 mg/kg significantly inhibited writhings compared to the control ($p<0.05$). (Fig 1). The percentage of inhibition of aspirin 200 mg/kg and AALE at doses of 100, 200 and 400 mg/kg were 55.32% and 35.65, 49.74, and 57.99%, respectively

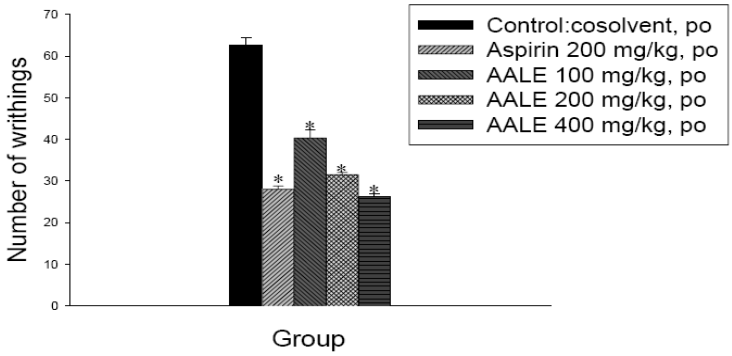

Figure 1: Effects of the n-hexane extract of *Abroma augusta* Linn**.** (AALE) and aspirin on acetic acid-induced writhing in mice. ***** $p<0.05$**,** significant different from control

Formalin test

The effects of AALE on early and late phase of the formalin test were shown in Fig 2. AALE at doses of 100, 200 and 400 mg/kg significantly inhibited both phases

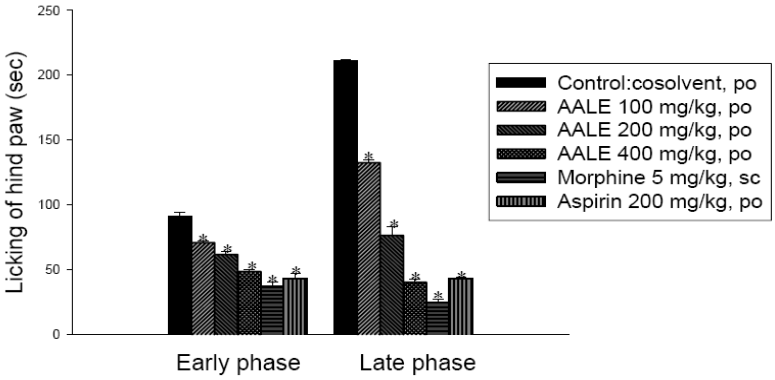

Figure 2: Effects of the n-hexane extract of *Abroma augusta* Linn**.** (AALE), morphine and aspirin on 2.5% formalin-induced paw licking in mice.
***** $p<0.05$**,** significant different from control

Hot plate test

AALE at doses of 100, 200 and 400 mg/kg increased in the reaction time to the thermal stimulus compared to control (Fig 3A). Naloxone (2 mg/kg, ip) give before the morphine (5 mg/kg, sc) or AALE (400 mg/kg, po) abolished the latency of the nociceptive response (Fig 3B).

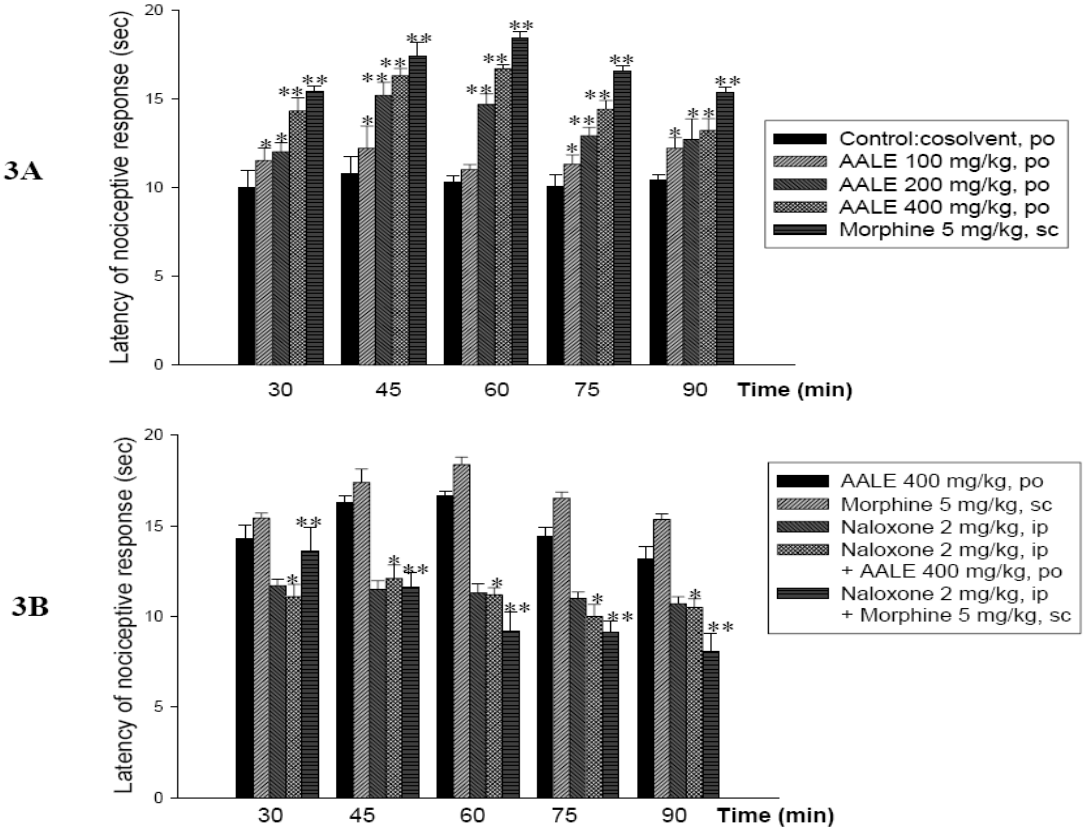

Figure 3 : Effects of AALE, morphine and naloxone on the reaction time of mice in the hot plate test. **3A**:***** $p<0.05$**,**** $p<0.01$,significant different from control; **3B**: ***** $p<0.01$**,** significant different from AALE 400 mg/kg , ****** $p<0.01$, significant different from morphine 5 mg/kg

Acute toxicity

AALE at the dose of 5 g/kg, po given to three male mice had no effect on their behavioral response and no mortality. The LD_{50} value of the extract in mice was therefore estimated to more than 5 g/kg, po. In this study, the doses of 100, 200 and 400 mg/kg, po given to mice is safe.

Discussion and conclusion

The hot plate and formalin tests have been used for investigation of centrally acting analgesic effect, whereas the acetic acid-induced method is widely used for the evaluation of peripheral antinociceptive activity (7). Many endogenous mediators such as substance P, histamine, serotonin, bradikinin and prostaglandind play an important role in the nociceptive mechanisms (8). The AALE at dose of 400 mg/kg marked significantly increased the latency time in hot plate test and decreased the licking time in formalin test. For the writhing test, the AALE at doses of 100, 200 and 400 mg/kg significantly inhibited writhing with dose-dependent manner. As the present evidence in this study, the antinociceptive activity of AALE was likely to have the mechanisms both the peripheral and central pathways.

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