Effects of Alkaloids Extracted from the Stem Bark of *Hunteria zeylanica* on Acute Inflammation in Experimental Animals

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The effects of crude alkaloids extracted from the stem bark of *Hunteria zeylanica* GARD. (*H. zeylanica*) on acute inflammatory responses such as carrageenin-induced paw edema in rats and croton oil- and arachidonic acid-induced ear edema in mice were investigated. Oral administration of *H. zeylanica* alkaloid extract (200–400 mg/kg) significantly suppressed the paw swelling induced by carrageenin. In the croton oil-induced ear edema, topical applied *H. zeylanica* alkaloid extract, at doses of 200 and 400 mg/ml, also significantly reduced ear edema. Moreover, the extract (50–200 mg/kg, p.o.) reduced in a dose-dependent manner the ear swelling induced by topical applied arachidonic acid (2 mg/ear). These results suggest that the inhibitory effects of *H. zeylanica* alkaloid extract on acute edema formation are partly due to inhibition of 5-lipoxygenase and cyclooxygenase activity.

**Keywords**  *Hunteria zeylanica*; acute inflammation; paw edema; ear edema; cyclooxygenase; 5-lipoxygenase

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*Hunteria zeylanica* GARD. (*H. zeylanica*) is a glabrous tree of the family Apocynaceae. This plant has been used not only as a folk medicine for treatment of yaws but also as a substitute for kemuning (Murraya paniculata, Rutaceae), which is used to reduce boils and skin irritations. In our previous study, we found that *H. zeylanica* alkaloid extract exhibited antinociceptive and antipyretic effects in mice and rats. Furthermore, this alkaloid extract inhibited the vascular permeability produced by acetic acid in mice. These findings suggest that the extract possesses anti-inflammatory properties. However, to our knowledge, no reports have been published on its anti-inflammatory action. In the present study, we investigated the effects of *H. zeylanica* alkaloid extract on acute inflammatory responses using several experimental models such as carrageenin-induced paw edema in rats and croton oil- and arachidonic acid-induced ear edema in mice.

**MATERIALS AND METHODS**

**Materials**  Authentication of the stem bark of *H. zeylanica* GARD. was achieved by comparison with herbarium specimens in the Department of Biology, Faculty of Sciences, Prince of Songkla University, Thailand.

**Plant Extract**  A crude alkaloid fraction was prepared from the stem bark of *H. zeylanica* GARD. as previously described. Briefly, the dried coarsely-powdered stem bark of this plant (1.5 kg) was moistened with 25% ammonia solution and allowed to stand overnight. It was then macerated with 95% ethanol (7 l) for 3 d and filtered. The residue was macerated again with 95% ethanol (7 l) for 2 d and then filtered; this step was repeated three times. The combined filtrates were concentrated to a syrupy mass under reduced pressure and then combined with 2% sulfuric acid solution (10 × 100 ml). After shaking and filtering, the acidic filtrate was washed with portions of benzene (2 × 100 ml), made basic (pH 10) with 25% ammonia solution, and then extracted with portions of chloroform (12 × 100 ml). The combined chloroform extracts were washed with water (2 × 200 ml), dried over anhydrous sodium sulfate and evaporated to yield a coarse brown powder of crude alkaloids (20 g).

**Animals**  All animals used in this study were obtained from Japan SLC, Shizuoka, Japan. Male ddY mice weighing 26–35 g were used for all experiments except for the carrageenin-induced paw edema test which was carried out using male Wistar rats weighing 120–150 g. The rats were handled for 5–10 min daily for several days before data collection. Animals were housed for at least one week in the laboratory animal room before experiments were begun. Housing conditions were thermostatically maintained at 23 ± 1 °C with 60% humidity, with a 12:12 light-dark cycle. Food and water were given *ad libitum*.

**Carrageenin-Induced Paw Edema in Rats**  According to the method described by Winter et al., the initial right hindpaw volume of the rats was measured using a volume meter (MK-550, Muromachi) and then 0.1 ml of 1% carrageenin was subcutaneously injected into the subplantar region of the right hindpaw. The volume of right hindpaw was measured at 0.5, 1, 2, 3, 4, and 5 h after carrageenin injection, and the edema volume was determined. The data were expressed as a percentage swelling, compared with the initial hindpaw volume of each rat. Vehicle, *H. zeylanica* alkaloid extract or aspirin was orally administered 30 min before carrageenin injection.

**Croton Oil Ear Edema in Mice**  The method modified by Tonelli et al. and Ucelay et al. was used. Briefly, mice were anesthetized with ether, and either mineral oil or test agents were topically applied to both sides of the right ear of the mouse in a volume of 0.05 ml and then

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0.05 ml of 100% croton oil was topically applied to the same area 15 min after the test agents. Six hours later the animals were sacrificed and the right ear was cut, punched out with a cork borer (7 mm in diameter) and weighed. The left ear without the irritant was used as a blank and the difference in weight between the right and left ears was taken as the index of anti-inflammatory activity of the test agents. Test agents were completely dissolved in mineral oil before the experiments were carried out.

**Arachidonic Acid-Induced Ear Edema in Mice**

According to the method reported by Young et al.,10 arachidonic acid dissolved in acetone (100 mg/ml) was applied in a volume of 0.02 ml to the inner and outer surfaces of the right ear (2 mg/ear) of the mouse under ether anesthesia, and then dried with a hand-held hair dryer. Vehicle or test agents were given orally 30 min before arachidonic acid application. One hour after arachidonic acid, animals were sacrificed by cervical dislocation and the ears were quickly cut, punched out with a cork borer (7 mm in diameter) and weighed. The left ear without arachidonic acid was used as a blank and the difference in weight between the right and left ears was taken as the index of anti-inflammatory activity of the test agents.

**Drugs**

The following drugs were used: aspirin (Iwaki Seiyaku, Tokyo, Japan), triamcinolone, carboxymethyl cellulose sodium salt (Nacalai Tesque, Inc., Kyoto, Japan), carrageenin (Piccin-A®, Lambda type, Zushikagaku Laboratory, Kanagawa, Japan), arachidonic acid, phenidone (Sigma Chem. Co., St. Louis, U.S.A.), croton oil (Wako Pure Chem. Ltd., Osaka, Japan), and mineral oil (Kanto Chem. Co., Inc., Tokyo, Japan). H. zeylanica alkaloid extract, aspirin, and phenidone were suspended in 0.5% sodium carboxymethyl cellulose (CMC) solution, and administered orally in a constant volume (10 ml/kg for mice and 5 ml/kg for rats) except when otherwise specified. All drug solutions were prepared immediately before the start of the experiments.

**Statistical Analysis**

The data from the carrageenin-induced edema experiment were analyzed using a parametric two-way repeated measures analysis of variance (ANOVA) followed by the Student–Newman–Kuels test. The data from the arachidonic acid-induced ear edema experiment were analyzed using a parametric one-way ANOVA followed by Dunnett’s test. The data from the croton oil-induced ear edema experiment were examined using the non-parametric Kruskal–Wallis analysis of variance followed by the Mann–Whitney U-test for multiple comparisons between groups. A difference was considered statistically significant at p < 0.05.

**RESULTS**

**Effect of H. zeylanica on Carrageenin-Induced Paw Edema in Rats**

As shown in Fig. 1, subplantar injection of 1% carrageenin produced paw edema in rats (F(5, 125) = 162.63, p < 0.001). The edema volume peaked 3 to 4 h after carrageenin injection. The paw volume in the vehicle control group increased by approximately 62% at 4 h after carrageenin injection. Significant interaction between drug treatment and time was observed (F(20, 125) = 6.322, p < 0.001). Oral administration of *H. zeylanica* alkaloid extract (200–400 mg/kg) significantly reduced the edema induced by carrageenin. The reference drug aspirin (400 mg/kg, p.o.) also significantly reduced the carrageenin-induced paw edema.

**Effect of H. zeylanica on Croton Oil-Induced Ear Edema in Mice**

Topically applied *H. zeylanica* extract, at doses of 200 and 400 mg/ml, produced a significant suppression of ear swelling induced by croton oil (Table I). The

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**Table I. Effect of H. zeylanica Alkaloid Extract, Aspirin and Triamcinolone on Croton Oil-Induced Ear Edema in Mice**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/ml)</th>
<th>n</th>
<th>Ear swelling (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral oil</td>
<td>—</td>
<td>25</td>
<td>6.1 (5.6–7.5)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>400</td>
<td>8</td>
<td>5.5 (5.0–7.0)</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>5</td>
<td>9</td>
<td>3.7 (2.9–5.2)</td>
</tr>
<tr>
<td><em>H. zeylanica</em> extract</td>
<td>100</td>
<td>10</td>
<td>2.2 (1.9–2.5)*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>10</td>
<td>2.1 (1.5–2.9)*</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>10</td>
<td>2.0 (1.4–2.8)*</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. * p < 0.05 compared with the vehicle control group (Mann–Whitney U-test).

**Table II. Effects of H. zeylanica Alkaloid Extract, Aspirin and Phenidone on Arachidonic Acid-Induced Ear Edema in Mice**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>Ear swelling (mg)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% CMC</td>
<td>—</td>
<td>20</td>
<td>12.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>200</td>
<td>10</td>
<td>12.0 ± 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>9</td>
<td>9.0 ± 0.7*</td>
<td></td>
</tr>
<tr>
<td>Phenidone</td>
<td>50</td>
<td>10</td>
<td>10.3 ± 0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>5.8 ± 0.3*</td>
<td></td>
</tr>
<tr>
<td><em>H. zeylanica</em> extract</td>
<td>50</td>
<td>10</td>
<td>10.0 ± 0.4*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>9.0 ± 0.4*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>10</td>
<td>6.7 ± 0.5*</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. * p < 0.05 compared with the vehicle control group (Dunnnett’s test).
reference drug triamcinolone, at a concentration of 10 mg/ml, also significantly suppressed the ear swelling, while aspirin had no effect at 400 mg/ml.

**Effect of H. zeylanica on Arachidonic Acid-Induced Ear Edema in Mice** Oral administration of the alkaloid extract (50—200 mg/kg) significantly suppressed the ear swelling induced by topicaly applied arachidonic acid in a dose-dependent manner (Table II). The reference drug phenidone, a dual inhibitor of cyclooxygenase and 5-lipoxygenase, also significantly reduced the edema at doses of 100 and 200 mg/kg. On the other hand, the cyclooxygenase inhibitor aspirin, at 200 mg/kg, did not affect the edema caused by arachidonic acid; it required a higher dose (400 mg/kg, p.o.) to produce significant suppression of the edema.

**DISCUSSION**

The present study demonstrated that *H. zeylanica* alkaloid extract was effective in animal models of acute inflammation. Systemic and topical administration of the extract significantly attenuated carrageen-induced paw edema in rats and croton oil-induced ear edema in mice, respectively. Furthermore, when given orally, it also suppressed the ear edema induced by arachidonic acid in mice.

The carrageen-induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effects of non-steroidal anti-inflammatory drugs which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis. The edema formation in this model can also be inhibited by dual cyclooxygenase/5-lipoxygenase inhibitors, but not by 5-lipoxygenase inhibitors. In the present study, oral administration of *H. zeylanica* alkaloid extract (200—400 mg/kg), as well as a cyclooxygenase inhibitor aspirin, significantly inhibited the paw edema formation induced by carrageenin, although the extract was less effective than aspirin. These findings suggest that the effect of the alkaloid extract on carrageen-induced inflammation is mediated by cyclooxygenase inhibition.

Croton oil-induced ear edema is a useful model for testing topical anti-inflammatory activity. In this study, topicaly-applied *H. zeylanica* alkaloid extract, as well as a reference steroidal drug triamcinolone, significantly reduced the inflammatory response caused by croton oil, indicating that the alkaloid extract can be absorbed through the cutaneous layers of the edematous area to exhibit anti-inflammatory action. In contrast to these agents, topicaly applied aspirin did not show any significant effect, even at a high dose (20 mg/ear). This result disagrees with the data presented by Van Arman that the ED50 of aspirin was 4.74 mg per ear in croton oil-induced ear edema. The discrepancy between his data and ours remains unclear, but may be due to the differences in the application method, dose of irritant, and/or the animal strains used. Nevertheless, the present results indicate that topical application of *H. zeylanica* alkaloid extract to the affected area is useful for the treatment of cutaneous inflammation.

Arachidonic acid-induced ear edema has been demonstrated to be a sensitive test for agents capable of inhibiting 5-lipoxygenase activity. Moreover, this inflammation reaction appears to be mainly mediated by the sulfidopeptide-leukotrienes, LTC4 and LTD4. In this study, *H. zeylanica* alkaloid extract, as well as a cyclooxygenase/5-lipoxygenase inhibitor phenidone, suppressed the edematous response caused by arachidonic acid in a dose-dependent manner, while the cyclooxygenase inhibitor aspirin had no effect at 200 mg/kg. Taken together, these results suggest that inhibition of 5-lipoxygenase activity is involved in the anti-inflammatory action of the extract on the arachidonic acid-induced edema. Although the inability of 200 mg/kg aspirin to suppress edema in this model is consistent with the data reported by Carlson et al., aspirin at 400 mg/kg slightly, but significantly, attenuated arachidonic acid-induced ear edema in this study. The mechanism of action of aspirin at this dose remains unclear.

*H. zeylanica* alkaloid extract given orally produced significant suppression in both acute inflammation models of carrageen-induced paw edema in rats and arachidonic acid-induced ear edema in mice, suggesting that the extract acts like a dual cyclooxygenase/5-lipoxygenase inhibitor *in vivo*. Nevertheless, the present data do not exclude the possible involvement of other factors independent of these enzymes in the anti-inflammatory activity of *H. zeylanica* extract, since other inflammatory mediators, such as histamine and serotonin, also reportedly play important roles in carrageen-induced paw edema and arachidonic acid-induced ear edema.

In conclusion, the present results indicate that *H. zeylanica* alkaloid extract can inhibit acute edematous formation, and suggest that the anti-inflammatory action of the extract is partly due to inhibition of 5-lipoxygenase and cyclooxygenase activity. To clarify the mechanisms of action of this plant extract will require measurement of arachidonic acid metabolism and investigations of the active principle(s) responsible for its anti-inflammatory action.

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