Antinociceptive and Antipyretic Effects of Alkaloids Extracted from the Stem Bark of *Hunteria zeylanica*

Wantana Reanmongkol, Kinzo Matsumoto, Hiroshi Watanabe, Sanan Subhadhirasakul, and Shin-Ichiro Sakai

Division of Pharmacology, Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University,* Toyama 930-01, Japan, Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand, and Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chiba University, Chiba 263, Japan.

Received May 2, 1994; accepted June 23, 1994

Effects of crude alkaloids extracted from the stem bark of *Hunteria zeylanica* GARD. (*H. zeylanica*) on nociceptive responses, capillary permeability, yeast-induced hyperthermia, pentobarbital-induced sleep, and spontaneous motor activity were investigated. Oral administration of 50 mg/kg *H. zeylanica* alkaloid extract significantly decreased the number of writhings induced by intraperitoneal acetic acid. The extract at 100—200 mg/kg significantly increased nociceptive threshold of the inflamed but not the non-inflamed paw in the Randall-Selitto test. Moreover, in the formalin test, the extract (100 mg/kg) significantly decreased licking activity in the late phase without affecting the activity in the early phase. However, the extract did not produce antinociceptive effect in the hot plate test, while it inhibited increase of vascular permeability induced by acetic acid in the capillary permeability test. Moreover, the extract dose-dependently reduced yeast-induced hyperthermia in rats without affecting normothermia. It did not affect pentobarbital-induced sleep, but significantly increased locomotor activity at 100 mg/kg. These results suggest that *H. zeylanica* alkaloid extract possesses antinociceptive and antipyretic effects, and that the former effect may be mediated by its anti-inflammatory action.

**Keywords** *Hunteria zeylanica*; antinociceptive activity; capillary permeability; antipyretic activity; anti-inflammatory action

*Hunteria zeylanica* GARD. (*H. zeylanica*) is a glabrous tree of the family Apocynaceae. The latex of this plant has been used as a folk medicine for treatment of the sores of yaws. The leaves and stem bark of *H. zeylanica* are known to contain alkaloids, although there are differences in the alkaloidal contents depending on the area where the plants were collected. Chemical constituents of the plant extract have been investigated, and some of them identified. However, there is no information available on the pharmacological actions of the alkaloids of this plant. In the present study, in order to preliminarily characterize pharmacological actions of the alkaloidal extract prepared from the stem bark of *H. zeylanica* GARD., we investigated effects of the extract on nociceptive responses in writhing test, hot plate test, formalin test and Randall-Selitto test, capillary permeability, yeast-induced hyperthermia, pentobarbital-induced sleep and spontaneous motor activity using aspirin and morphine as standard drugs.

**MATERIALS AND METHODS**

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

**Plant Extract** The dried coarsely powdered stem bark of *H. zeylanica* GARD. (1.5 kg) was moistened with 25% ammonia solution and allowed to stand overnight. It was macerated with 95% ethanol (7 l) for 3 d and then filtered. The residue was remacerated with 95% ethanol (7 l) for 2 d and filtered again; this step was repeated three times.

The combined filtrate was concentrated to a syrupy mass under reduced pressure, and then mixed with 2% sulfuric acid solution (100 ml × 10 times). After shaking and filtering, the acidic filtrate was washed with portions of benzene (100 ml × 2 times), made basic (pH 10) with a 25% ammonia solution, and then extracted with portions of chloroform (100 ml × 12 times). The combined chloroform extract was washed with water (200 ml × 2 times), dried with anhydrous sodium sulfate and evaporated to yield 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 the Randall-Selitto test and the yeast-induced fever test. Animals were housed for at least one week in the laboratory animal room before experiments. In the Randall-Selitto test, male Sprague-Dawley rats weighing 120—150 g were used. The rats were handled for 5—10 min daily for several days prior to data collection. Male Wistar rats weighing 130—160 g were used in the yeast-induced fever test. Housing conditions were thermostatically maintained at 23 ± 1°C with 60% humidity, at a 12:12 light-dark cycle. Food and water were given *ad libitum*.

**Acute Toxicity** The 50% lethal dose of the *H. zeylanica* extract was estimated by “up- and down-method” using ddY mice. Doses were adjusted by a constant multiplicative factor: e.g., 1.5 for this experiment. The dose for each successive animal was adjusted up or down depending on the previous outcome.

**Antinociceptive Activities** 1) Writhing Test: When testing writhing behavior, 0.6% acetic acid solution (10 ml/kg body weight) was intraperitoneally injected and the number of writhings and stretchings was counted over
a 20-min period as previously reported.8,9) Either the *H. zeylanica* alkaloid extract (25, 50 and 100 mg/kg), a reference antinociceptive drug aspirin (400 mg/kg) or control vehicle was orally administered 30 min before acetate. The inhibition by an antinociceptive agent was determined for each experimental group of 8 mice as follows:

\[ \% \text{ inhibition} = 100 \times (1 - \text{experimental/control}) \]

2) Hot Plate Test: The hot plate test was carried out according to the method described by Woolfe and MacDonald.10) Briefly, a mouse was placed on a hot plate maintained at \(55 \pm 1^\circ \text{C}\). The latency of nociceptive responses such as licking or jumping was measured. Thirty min after oral administration of the test agents (50—200 mg/kg *H. zeylanica* alkaloid extract) except morphine (5 and 10 mg/kg, 15 min after administration), the measurement was repeated every 15 min over a 60-min period. Morphine HCl was subcutaneously injected. The cut-off time was 45 s. Only the mice that showed nociceptive responses within 15 s before drug administration were used for the experiments.

3) Formalin Test: Thirty min after administration of the *H. zeylanica* alkaloid extract (25, 50 and 100 mg/kg, p.o.), aspirin (400 mg/kg, p.o.) or control vehicle, 20 μl of 5% formalin in saline was subcutaneously injected to a hindpaw of mice. The time spent licking the injected paw was recorded and the data were expressed as total licking time in the early phase (0—10 min) and the late phase (10—60 min) after formalin injection.11)

4) Randall-Selitto Paw Pressure Test in Rats: According to the method reported by Randall and Selitto,12) pressure was applied to each hindpaw of rats using an analgometer (Ugo Basile, Italy). Inflammation was caused by subcutaneously injecting 0.1 ml of 1% carrageenan suspension into the plantar region of the right hindpaw. After 2 h, either the *H. zeylanica* alkaloid extract (50, 100 and 200 mg/kg), aspirin (400 mg/kg) or 0.5% sodium carboxymethyl cellulose (CMC) was orally administered. The nociceptive threshold was defined as the pressure causing the animals to struggle, turn to bite and/or vocalize, and it was recorded at 1, 2 and 3 h after drug administration.

**Capillary Permeability Test** This test was performed according to the method of Whittle.13) In brief, 30 min after administration of control vehicle, the *H. zeylanica* alkaloid extract (50—200 mg/kg) or a reference drug aspirin (400 mg/kg), each mouse was intravenously injected with 0.2 ml of 0.25% (w/v) Evans blue solution. After 15 min, 0.6% acetic acid (10 ml/kg) was injected intraperitoneally. Thirty min after acetic acid, the mice were killed by dislocation of the neck. The viscera were rapidly exposed and irrigated with 7.0 ml saline over a petri dish. The combined washings were centrifuged at 3000 rpm for 10 min and the absorbance at 610 nm was measured using a UV-spectrophotometer (Shimadzu UV-240, Japan). The amount of dye was expressed as μg per 30 g body weight.

**Antipyretic Activity in Rats** Antipyretic activity of drug was measured by slightly modifying the method described by Adams *et al.*14) Briefly, male Wistar rats were fasted overnight with water *ad lib.* before the experiments. Pyrexia was induced by subcutaneously injecting 20% brewer’s yeast suspension (10 ml/kg) into the animals’ dorsum region. Seventeen hours after the injection, the rectal temperature of each rat was measured using a digital thermometer (Delta SK-1250MC, Sato Keiryoki Mfg. Co., Ltd.). Only rats that showed an increase in temperature of at least 1°C were used for the experiments. Test agents or control vehicle was orally administered and the temperature was again measured at 0.5, 1, 3 and 5 h after drug administration. When examining the effect of drugs on normothermia in rats, saline was injected subcutaneously instead of the yeast.

**Pentobarbital-Induced Sleep** Pentobarbital (50 mg/kg) was intraperitoneally injected to mice. The duration of sleep was measured as the period between the loss and the recovery of the righting reflex. Control vehicle, alkaloid extract of *H. zeylanica* (12.5—100 mg/kg) and a reference drug aspirin (400 mg/kg) were administered orally 30 min before pentobarbital.15)

**Spontaneous Motor Activity** Animate (MATYS, Toyama, Japan) was used to measure spontaneous motor activity in mice as described previously.16) To habituate a mouse to a new surrounding, the animal was placed in a doughnut-shaped cage 30 min before the experiments. Either control vehicle or the *H. zeylanica* alkaloid extract (25—100 mg/kg) was orally administered. After 30 min, changes in spontaneous motor activity were recorded during a 30-min period. Experiments were carried out from 9 A.M. to 5 P.M.

**Drugs** The following drugs were used: pentobarbital sodium (Tokyo Kasei Kogyo, Tokyo, Japan), morphine HCl (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan), aspirin (Iwaki Seiyaku Tokyo, Japan), Evans blue, acetic acid, carboxymethyl cellulose sodium salt and formalin (NacalaiTesque, Inc., Kyoto, Japan), carrageenin (Lambda type) and brewer’s yeast (Sigma Chemical Co., St. Louis, U.S.A.). *H. zeylanica* alkaloid extract and aspirin were suspended in 0.5% sodium CMC solution. Other chemicals were dissolved in saline except for specifically stated cases. All drug solutions were prepared immediately before the experiments, and administered in a constant volume (10 ml/kg for mice and 5 ml/kg for rats) 30 min before the experiments.

**Statistics** Parametric data were analyzed with one-way analysis of variance (ANOVA) followed by Dunnnett’s test. Non-parametric data were analyzed with the 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**

The LD50 values of intraperitoneally and orally administered *H. zeylanica* alkaloid extract in mice were 72.4 mg/kg and 1.4 g/kg, respectively.

**Effects of *H. zeylanica* on Nociceptive Responses**

**Writhing Test:** Oral administration of the *H. zeylanica* alkaloid extract (50 mg/kg) significantly attenuated the writhing behavior induced by intraperitoneal 0.6% acetic acid (Table I). The reference drug aspirin (400 mg/kg) also
exhibited a significant protective effect on the acetic acid-induced writhing behavior.

Hot Plate Test: The mean reaction time to thermal stimuli is summarized in Table II. The H. zeylanica alkaloid extract (50, 100 and 200 mg/kg, p.o.) did not significantly change the latency to show nociceptive responses in the hot plate test in mice. In contrast, the reference drug morphine (5 and 10 mg/kg, s.c.) dose-dependently prolonged the latency of nociceptive responses.

Formalin Test: The H. zeylanica alkaloid extract did not alter the duration of licking activity in the early phase, while at a dose of 100 mg/kg the extract significantly reduced the duration in the late phase (Fig. 1). In contrast, a reference antinociceptive drug aspirin (400 mg/kg) also significantly decreased licking activity in both phases.

Randall-Selitto Test in Rats: As shown in Fig. 2, the reference drug aspirin (400 mg/kg) raised the maximum threshold for the nociceptive response caused by compression of the carrageenan-inflamed paw without affecting the response of the non-inflamed paw. Oral administration of the H. zeylanica alkaloid extract (100—200 mg/kg) also significantly raised the threshold

---

**Table I. Effect of H. zeylanica and Aspirin on Acetic Acid-Induced Writhing in Mice**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg, p.o.)</th>
<th>No. of writhings (counts/20 min)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% CMC</td>
<td>—</td>
<td>31.1 ± 2.3</td>
<td>0</td>
</tr>
<tr>
<td>H. zeylanica</td>
<td>25</td>
<td>29.8 ± 2.6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>21.1 ± 2.2*</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>22.5 ± 1.9*</td>
<td>28</td>
</tr>
<tr>
<td>Aspirin</td>
<td>400</td>
<td>17.5 ± 3.1**</td>
<td>44</td>
</tr>
</tbody>
</table>

Each value is the mean ± S.E.M. of 8 mice. * p<0.05, ** p<0.01, compared to 0.5% CMC (Dunnett's test).

---

**Table II. Effect of H. zeylanica and Morphine HCl on Nociceptive Response Induced by Heat in Mice**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Latency of nociceptive responses (s)</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% CMC</td>
<td>—</td>
<td>4.5 (3.0—6.0)</td>
<td>3.5 (1.0—5.0)</td>
<td>4.5 (2.0—5.0)</td>
<td>4.0 (1.0—5.0)</td>
<td></td>
</tr>
<tr>
<td>H. zeylanica</td>
<td>50</td>
<td>2.5 (2.0—3.0)</td>
<td>2.5 (2.0—6.0)</td>
<td>3.0 (1.0—4.0)</td>
<td>1.5 (1.0—4.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>7.0 (3.0—12.0)</td>
<td>2.5 (2.0—7.0)</td>
<td>5.0 (1.0—10.0)</td>
<td>1.5 (1.0—3.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>4.0 (2.0—12.0)</td>
<td>2.0 (2.0—7.0)</td>
<td>1.5 (1.0—3.0)</td>
<td>1.0 (1.0—3.0)</td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td>5</td>
<td>12.0 (8.0—17.0)**</td>
<td>14.5 (13.0—22.0)**</td>
<td>11.5 (8.0—16.0)**</td>
<td>10.0 (9.0—10.0)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>16.0 (15.0—20.0)**</td>
<td>23.5 (22.0—45.0)**</td>
<td>29.5 (17.0—31.0)**</td>
<td>14.0 (13.0—17.0)**</td>
<td></td>
</tr>
</tbody>
</table>

Each value is the median with 25—75 percentiles obtained from 10 mice. Latency of nociceptive responses was measured before and after drug administration. Test agents except morphine were orally administered 30 min before measurement of nociceptive responses. Morphine was subcutaneously injected 15 min before test. ** p<0.01 compared with control values (Mann-Whitney U-test).

---

**Fig. 1. Effect of H. zeylanica Alkaloid Extract and Aspirin (ASA) on Hindpaw Licking in the Formalin Test in Mice**

(A) Time course of paw-licking after formalin injection. Thirty min after drug administration (p.o.), 5% formalin was subcutaneously injected to a hindpaw in a volume of 20 μl at time 0. Each point represents the median value of time animals spent licking during a 5-min observation period. (B) Effect of H. zeylanica and aspirin on the paw-licking in the early (left) and late phases (right; 10—60 min after formalin injection). Each column represents the median with 25—75 percentiles (n=8). * p<0.05 and ** p<0.01, compared to the control group (Mann-Whitney U-test). ○, 0.5% CMC; □, H. zeylanica 25 mg/kg; △, H. zeylanica 50 mg/kg; ●, H. zeylanica 100 mg/kg; ▲, ASA 400 mg/kg.
Fig. 2. Antinociceptive Activity of *H. zeylanica* Alkaloid Extract and Aspirin in the Randall-Selitto Test in Rats

Carageenin was subcutaneously injected to the right hindpaw 2 h before the experiments. Either vehicle, *H. zeylanica* extract, or aspirin was orally administered at time 0, and the nociceptive thresholds of the inflamed (A) and non-inflamed paw (B) were measured at 1-h intervals for 3 h. Each point represents the mean ± S.E.M. of 6 rats. *p < 0.05 compared to the control group (Dunnett’s test). —□—, 0.5% CMC; —■—, aspirin 400 mg/kg; —○—, *H. zeylanica* 50 mg/kg; —●—, *H. zeylanica* 100 mg/kg; —△—, *H. zeylanica* 200 mg/kg.

Fig. 3. Effect of *H. zeylanica* Alkaloid Extract on Capillary Permeability in Mice

Control vehicle, the *H. zeylanica* extract or aspirin was orally administered. Each value indicates the median value with 25—75 percentiles (n = 9—15). *p < 0.05 and **p < 0.01 compared to the control group (Mann-Whitney U-test).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg, p.o.)</th>
<th>(A) Pentobarbital-induced sleep (min)</th>
<th>(B) Locomotor activity (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% CMC</td>
<td></td>
<td>60.4 ± 3.9</td>
<td>129 (0—881)</td>
</tr>
<tr>
<td><em>H. zeylanica</em></td>
<td>12.5</td>
<td>72.1 ± 6.2</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>76.8 ± 5.7</td>
<td>1089 (0—3009)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>84.5 ± 9.2</td>
<td>821 (181—2735)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>68.5 ± 4.4</td>
<td>1357 (949—1583)*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>400</td>
<td>65.5 ± 4.7</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

(A) *H. zeylanica* alkaloid extract and aspirin were orally administered. After 30 min, pentobarbital (50 mg/kg, i.p.) was injected, and sleeping time was measured. Each datum represents the mean ± S.E.M. from 8 mice. (B) Thirty min after *H. zeylanica* administration (p.o.), changes in spontaneous motor activity were measured over a 30-min period. Each datum represents the median with 25—75 percentiles from 10 mice. *p < 0.05 compared to the control group (Mann-Whitney U-test). n.d., not determined.

for the nociceptive response of the inflamed but not the non-inflamed paw.

**Effect of *H. zeylanica* on Increase of Capillary Permeability**

Permeability Induced by Acetic Acid: The *H. zeylanica* alkaloid extract (200 mg/kg, p.o.) produced a significant inhibitory effect on acetic acid-increased capillary permeability (Fig. 3).

**Effect of *H. zeylanica* on Yeast-Induced Hyperthermia in Rats** The *H. zeylanica* alkaloid extract dose-dependently reversely yeast-induced hyperthermia and the effect peaked 2 to 3 h after administration (Fig. 4). A reference drug aspirin also dose-dependently reversed hyperthermia induced by yeast. However, neither aspirin
nor the extract induced hypothermia in rats that received saline instead of yeast suspension.

Effect of *H. zeylanica* on Pentobarbital-Induced Sleep and Locomotor Activity in Mice Neither *H. zeylanica* alkaloid extract nor a reference antinociceptive drug, aspirin, altered the sleeping time (Table III). The extract significantly increased spontaneous locomotor activity at 100 mg/kg. However, the effect was not dependent on the dose tested.

DISCUSSION

The results demonstrated that crude alkaloids extracted from *H. zeylanica* produced antinociceptive actions on chemical and mechanical stimuli. This extract attenuated nociceptive responses in the acetic acid-induced writhing test. Furthermore, in the Randall-Selitto test this extract raised the pain threshold of the inflamed paw without affecting the threshold of the normal paw. The centrally acting antinociceptives reportedly affect the nociceptive threshold of the inflamed paw as well as the normal paw, while peripheral antinociceptives only affect the threshold of the inflamed paw. Therefore, the present findings suggest that peripheral mechanisms are involved in the antinociceptive action of the *H. zeylanica* alkaloid extract. This idea seems to be further supported by the fact that the extract failed to prolong the latency to show nociceptive responses in the hot plate test, since centrally but not peripherally acting antinociceptive compounds are known to prolong the latency in this test.

It is possible that sedative action produces the antinociceptive effect in the tests used in this study. However, this does not seem to be the case, since the *H. zeylanica* alkaloid extract did not affect pentobarbital-induced sleep or decrease locomotor activity in mice. Rather, the extract significantly increased the locomotor activity, though the effect was not dose-dependent.

When effects on capillary permeability were examined, the *H. zeylanica* alkaloid extract at 200 mg/kg significantly reduced leakage of Evans blue dye caused by intra-peritoneal acetic acid. The inhibitory action suggests that *H. zeylanica* alkaloids have anti-inflammatory action, and that this anti-inflammatory effect may participate in the nociceptive responses in the Randall-Selitto test. This idea seems to be further supported by the finding in the formalin test, in which the effects of drugs on the licking responses in the early and late phases reportedly represent antinociceptive action on sensory receptor stimulation and anti-inflammatory action, respectively. The *H. zeylanica* alkaloid extract exhibited dose-dependent reduction of licking activities in the late phase in this study without affecting the responses in the early phase, suggesting the anti-inflammatory action of this alkaloid extract. On the other hand, consistent with the data reported by Hunskaar and Hole, aspirin, a reference drug, significantly reduced the licking responses in not only the early phase but also the late phase. The antinociceptive effects of aspirin in the early and late phases in the formalin test appear due to central action and anti-inflammatory action mediated by inhibition of prostaglandin synthesis, respectively.

The *H. zeylanica* alkaloid extract as well as aspirin reversed yeast-induced hyperthermia without affecting normothermia in rats. The antipyretic action of aspirin also appears to involve its inhibitory effect on prostaglandin synthesis. In the present study, the antipyretic action of aspirin was observed at the same doses as the antinociceptive action, whereas the effective dosages of the extract to produce such an antipyretic action were higher than those to produce antinociceptive effects. Thus, these findings give rise to at least two possibilities. First, the antinociceptive action of the *H. zeylanica* alkaloid extract may be mediated by mechanisms differing from those governing the antipyretic action of the extract. Secondly, the alkaloidal constituents which produce the antinociceptive action may differ from those involved in the antipyretic action. To clarify the mechanism of action will require further investigations.

The stem bark of *H. zeylanica* contains many kinds of indole alkaloids such as eburnamine and eburnamine derivatives. Recently, one of the authors (S. Subhadhirasakul) has identified (+)-eburnamine, (+)-eburnamine, (+)-isoeburnamine, and other components of the crude alkaloids fraction of *H. zeylanica* (unpublished data). Therefore, it is of interest to learn which constituents contribute to the apparent antinociceptive action of the *H. zeylanica* alkaloid extract. These experiments are currently in progress in this laboratory.

In conclusion, the present results suggest that the *H. zeylanica* alkaloid extract possesses antinociceptive and antipyretic effects and that the former effect may be mediated by the extract’s anti-inflammatory action.

REFERENCES AND NOTES

1) W. Reanmongkol is recipient of a scholarship from the Ministry of Education, Sciences and Culture, Japan.


