Attenuation of Persistent Pain and Hyperalgesia by *Spilanthes acmella* Flowers in Rats

W.D. Ratnasooriya¹ and K.P.P. Pieris²

¹Department of Zoology, University of Colombo, Colombo, Sri Lanka; ²Department of Surgery and E.N.T., Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Colombo, Sri Lanka

**Abstract**

The effectiveness of *Spilanthes acmella* Murr. (Compositae) flowers against continuous inflammatory pain and its anti-hyperalgesic potential were investigated in vivo. Rats were administered a cold-water extract (CWE) in three different doses: 500, 1000, or 1500 mg/kg given orally. Pain reduction was measured with the formalin test of nociception and carrageenan-induced thermal hyperalgesia test. In the formalin test, the CWE significantly (p < 0.05) impaired the number of paw lickings and the time spent on paw licking in both early and late phases. In the carrageenan hyperalgesia test, CWE markedly and significantly (p < 0.05) prolonged the hot-plate response latency from ½ h up to 6 h after treatment. The CWE possessed antihistaminic activity (determined by histamine wheal test) and inhibited prostaglandin synthesis (as judged from the impairment of frequency of contumous contraction in isolated dicesorous rat uterus) activities but had no membrane stabilization effect (in heat-induced rat erythrocyte hemolysis test) or antioxidant activities. It is concluded that CWE of *S. acmella* flowers possess antinociception activity against persistent pain and anti-hyperalgesic activity, possibly by inhibiting prostaglandin synthesis, interrupting nociception transmission, and exerting antihistaminic activity.

**Keywords:** Anti-hyperalgesia, antinociception, continuous pain, *Spilanthes acmella*.

**Introduction**

*Spilanthes acmella* Murr. (Compositae), commonly called *acmella* in Sinhala and *akkirakaram* in Tamil, is an annual or short-lived perennial herb, 20–60 cm tall with a prostrate or ascending branched cylindrical more or less hairy stem and simple ovate opposite leaves (2.5–4.5 cm long, 2–4 cm broad) without stipules. The flowers are seasonal (October to January), small yellow, nonfragrant with five petals on long glabrous peduncles (3.8–6.0 cm long) (Jayaweera, 1981). Phytochemically, flowers of *S. acmella* are reported to contain amino acids (Pieris et al., 2001), alkaloids (Pieris et al., 2001), and N-isobutylamides (spilanthol, undeca-2E,7Z,9E-trienoic acid isobutylamide, and undeca-2E-en-8,10 diynoic acid isobutyleomide) (Ramsewak et al., 1999). This herb is indegenous to India, Sri Lanka, and other tropical countries (Jayaweera, 1981). In Sri Lanka, it is common in moist places up to 1800 m altitude (Jayaweera, 1981). Sri Lankan traditional practitioners, especially in the Uva province, claim that cold infusions of the flowers of *S. acmella* have strong diuretic activity and also dissolve urinary calculi. The flowers are also chewed or used in the form of a tincture to relieve severe toothache (Jayasinge, 1994). Recently, we scientifically investigated the diuretic (Ratnasooriya et al., 2004) and antinociceptive potential (Pieris et al., 2001) of a cold-water extract (CWE) of *S. acmella* flowers in rats and found that CWE had biological activity that corresponded to the claimed activities. However, the experimental models we have used to evaluate antinociceptive activity in the previous study (Pieris et al., 2001) have a major limitation: the tests only evaluate the activity of CWE of the flower to impair phasic transient pain, not continuous inflammatory pain (Wong et al., 1994; Langerman et al., 1995) that is more important in clinical settings. Persistent pain is known to have a major impact on quality of life (i.e., physical, social, and emotional health).
Pain management by Spilanthes acmella

(Crook et al., 1984). Further, to our knowledge, the antihyperalgesic potential of S. acmella flowers is not scientifically documented.

The aim of this study was to investigate the effects of CWE of S. acmella flowers on acute moderate continuous inflammatory pain and its antihyperalgesic potential. This was done using formalin (Dubuisson & Dennis, 1977) and carrageenan-induced thermal hyperalgesia tests (Richardson et al., 1998), respectively, in rats.

Materials and Methods

Experimental animals

Healthy adults cross-bred albino rats (weighing 200–225 g) from our own colony were used. They were housed under standardized animal housing conditions and had free access to pelleted food (Master Feed Ltd., Colombo, Sri Lanka) and water. Animals were used only once.

Collection of flowers

Yellow-colored fresh flowers were collected from mature Spilanthes acmella plants at the Ayurvedic medicinal garden, Haldumulla (Uva Province), Sri Lanka, in June 2002. The identification and authentication was performed by Mr. S.B. Weerakoon, Department of Ayurveda (Colombo, Sri Lanka). A voucher specimen (Am/01, 2002) is deposited at the museum of the Department of Zoology, University of Colombo, Sri Lanka.

Preparation of the cold-water extract

Fresh flowers (482 g) were homogenized in distilled water (DW) (400 ml) using a domestic blender (National Model MX-T, Matsushita Ltd, Tokyo, Japan) at fast speed for 10 min and filtered through eight layers of muslin cloth. The resulting brown-colored filtrate was freeze-dried (17.5 g, yield 3.6% w/v) and stored air-tight at 4°C. The freeze-dried powder was dissolved in DW to obtain the required dosage concentration (in terms of fresh weight) to be administered orally in 1 ml solution (500, 1000, or 1500 mg/kg). These doses are identical to what has been used previously (Ratnasooriya et al., 2004).

Evaluation of antinociception activity against acute tonic pain using formalin test

This was performed as described by Farsam et al. (2000). Thirty rats were divided into four groups and were orally administered with the CWE or vehicle as follows: group 1 (n = 6) with low-dose 500 mg/kg of CWE; group 2 (n = 6) with mid-dose 1000 mg/kg of CWE; group 3 (n = 10) with high-dose 1500 mg/kg of CWE; group 4 (n = 8) with 1 ml of DW (vehicle). Three hours after administration, each rat was subcutaneously injected with 0.05 ml of 2.5% formalin solution (BDH Chemicals, Poole, UK) into the subplantar surface of the left hind paw. Rats were observed for 60 min and the number of lickings and the amount of time spent licking the injected paw were recorded in two phases: first phase, 1–5 min, and second phase, 15–60 min.

Evaluation of the antihyperalgesic activity

Evaluation of the antihyperalgesic activity was performed as described by Richardson et al. (1998). Fifty-four rats were assigned into 7 groups and orally treated in the following manner: group 1 (n = 6) with low-dose 500 mg/kg of CWE; group 2 (n = 6) with mid-dose 1000 mg/kg of CWE; group 3 (n = 12) with high-dose of CWE; group 4 (n = 24) with 0.5 ml of DW; and group 5 (n = 6) with 0.3 mg of morphine sulfate. Immediately afterwards, these rats were subcutaneously injected with 0.05 ml of 1% carrageenan suspension (Sigma Chemical Company, St. Louis, MO, USA) in normal saline into the plantar surface of the left hind paw to induce pain. Half an hour later, and then at hourly intervals for 6 h, the reaction time, at 50°C, of the injected paw (the time taken to lick) was assessed using the hot-plate technique (Model MK 35 A, Muromachi Kikai Co. Ltd., Tokyo, Japan).

Evaluation of prostaglandin synthesis inhibition activity

This was done as described by Dharmasiri et al. (2003). Briefly, nine female rats in diestrous were selected by microscopic examination of vaginal smears. They were sacrificed with an overdose of ether; their uterine horns were removed and cut into approximately 1-cm pieces. These uteri were individually placed in a 50-ml organ bath containing Kreb's Henseleit solution having the following composition (mmol/l): Na+: 143; K+: 5.8; Ca²⁺: 2.6; Mg²⁺: 1.2; Cl⁻: 128; H₂PO₄: 1.2; HCO₃⁻: 25; SO₄⁻: 1.2, and glucose 11.1 at a pH of 7.4. The organ bath was maintained at 37°C and aerated with a gas mixture of 95% O₂ and 5% CO₂. The spontaneous activity of the uterus was recorded isometrically under a resting tension of 1 g until the contractions became regular (usually within 10 min) using an isometric sensor (Star Medicals, Tokyo, Japan). After the contractions became regular, the normal activity of the uterus was recorded for a further 10 min. Following this, CWE was added sequentially into the organ bath so that the final concentrations became 2.5, 10, 30 μl/ml in the organ bath (n = 3/dose). After each treatment, the spontaneous activity of the uterus was further recorded for 20 min. The amplitude and frequency of contractions were calculated.

Evaluation of plasma membrane stabilization activity

This activity was evaluated using heat-induced hemolysis of rat erythrocytes in vitro as described by Dharmasiri
et al. (2003). The concentrations of CWE used were 12.5, 25, 50, and 100 μg/ml. Phosphate-buffered saline (pH 7.4) was used as control. The absorbance of the supernatant was measured at 540 nm using a spectrophotometer (JASCO V500, Jasco Corporation, Tokyo, Japan). The percentage inhibition of hemolysis with respect to the controls was calculated as follows:

\[
\text{% inhibition of hemolysis} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100
\]

**Evaluation of rectal temperature**

Twelve rats were randomly divided into two equal groups (n = 6/group). One group was orally administered with the highest dose of the CWE and the other with 1 ml of DW. Four hours later, their rectal temperature was measured using a clinical thermometer (TM-II, Normal glass, Focal Corporation, Tokyo, Japan).

**Evaluation of antihistaminic activity**

Fur on the left lateral side of the posterior region of 18 rats was shaved under aseptic conditions. Twenty-four hours later, these rats were randomly divided into three equal groups (n = 6/group). These rats were orally treated in the following manner. Group 1 with 1.5 ml of DW; group 2 with 0.67 mg/kg chlorpheniramine, an antihistamine drug (Rang et al., 1995); and group 3 with the high-dose of CWE. After 1 h, these rats were subcutaneously injected with 50 μl of 500 μg/ml of histamine dihydrochloride (Fluka, Buchs, Switzerland) in the shaved region, and 2 min later the area of the wheal formed was measured (Spector, 1956).

**Statistical analyses**

The results are given as means ± SEM. Statistical comparison was made using Mann-Whitney U-test. Significance was set at p ≤ 0.05.

**Results**

**Formalin test**

As shown in Table 1, all the three doses of CWE of S. aemella flowers significantly (p < 0.05) impaired the numbers of licking (first phase: low dose by 61%, mid-dose by 48%, and high-dose by 41%; second phase: low dose by 78%, mid-dose by 69%, and high-dose by 38%), and time spent on licking the injected paw (first phase: low-dose by 40%, mid-dose by 28%, and high-dose by 46%, second phase: low dose by 45%, mid-dose by 40%, and high-dose by 23%) in both phases. These effects were dose-related (number of lickings inversely: first phase \( r^2 = -0.95 \), p < 0.05 and second phase \( r^2 = -0.84, p < 0.05 \)) and time spent on licking curvilinearly (in first phase \( r^2 = 1.0, p < 0.05 \); in second phase \( r^2 = 0.81, p < 0.05 \)). Overall, there appears to be no marked difference in the effects seen between first and second phases.

**Antihyperalgesic activity**

Table 2 summarizes the results obtained. As shown in the hot-plate test, all three doses of CWE significantly (p < 0.05) prolonged the reaction time from \( \frac{1}{2} \) h up to 4 h after carrageenan injection. This CWE-induced antihyperalgesia was dose-related (for the \( \frac{1}{2} \) h: \( r^2 = 0.82, p < 0.05 \); for the first h: \( r^2 = 0.99, p < 0.05 \); for second h: \( r^2 = 0.97, p < 0.05 \); for the fourth h: \( r^2 = 1.0, p < 0.05 \); for fifth h: \( r^2 = 0.98, p < 0.05 \); and the sixth h: \( r^2 = 0.92, p < 0.05 \)). On the other hand, with morphine prolongation, the reaction time was about 1.5-fold higher than that of CWE up to 1 h.

**Prostaglandin synthesis inhibition activity**

The mean amplitude and frequency of isolated rat uterine strips were, respectively, 1.05 ± 0.20 g and 1.60 ± 0.33 contractions/min. As shown in Table 3, all the three concentrations of the CWE tested failed to inhibit the amplitude of the uterine contraction. In contrast, CWE

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**Table 1.** Effect of oral administration of cold-water extract (CWE) of Spilanthes aemella flowers on the licking frequency and time of rats in formalin test (means ± SEM).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Licking frequency (mean ± SEM)</th>
<th>Duration of each licking (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 8)</td>
<td>16.0 ± 1.5</td>
<td>9.4 ± 2.3</td>
</tr>
<tr>
<td>CWE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1500 mg/kg (n = 10)</td>
<td>9.5 ± 0.5*</td>
<td>5.1 ± 0.5*</td>
</tr>
<tr>
<td>1000 mg/kg (n = 6)</td>
<td>8.3 ± 1.5*</td>
<td>6.8 ± 1.2</td>
</tr>
<tr>
<td>500 mg/kg (n = 6)</td>
<td>6.3 ± 1.1*</td>
<td>5.6 ± 3.2*</td>
</tr>
</tbody>
</table>

* p < 0.05 as compared with control: number of animals used are given in parentheses.
Table 2. Effect of oral administration of cold-water extract (CWE) of *S. acmella* flowers on the reaction time of rats in the carrageenan-induced thermal hyperalgesia test (means ± SEM).

<table>
<thead>
<tr>
<th></th>
<th>Pretreatment</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>5 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 24)</td>
<td>5.7 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td>5.0 ± 0.2</td>
<td>5.3 ± 0.2</td>
<td>5.1 ± 0.2</td>
<td>5.0 ± 0.2</td>
<td>5.1 ± 0.2</td>
</tr>
<tr>
<td>CWE 1500 mg/kg (n = 12)</td>
<td>5.1 ± 0.3</td>
<td>7.7 ± 0.5*</td>
<td>8.4 ± 0.3*</td>
<td>8.2 ± 0.4*</td>
<td>7.6 ± 0.3*</td>
<td>6.8 ± 0.4</td>
<td>6.0 ± 0.3</td>
</tr>
<tr>
<td>1000 mg/kg (n = 6)</td>
<td>4.8 ± 0.4</td>
<td>7.6 ± 0.4*</td>
<td>10.0 ± 0.8*</td>
<td>10.0 ± 0.7*</td>
<td>8.2 ± 0.5*</td>
<td>8.4 ± 0.8</td>
<td>6.8 ± 0.6</td>
</tr>
<tr>
<td>500 mg/kg (n = 6)</td>
<td>5.6 ± 1.0</td>
<td>8.2 ± 0.3</td>
<td>9.1 ± 0.6*</td>
<td>8.2 ± 0.4*</td>
<td>7.8 ± 0.3*</td>
<td>6.2 ± 0.4</td>
<td>6.0 ± 0.3</td>
</tr>
<tr>
<td>0.3 mg morphine (n = 6)</td>
<td>5.4 ± 0.3</td>
<td>11.4 ± 0.7*</td>
<td>11.4 ± 1.2*</td>
<td>8.7 ± 0.9*</td>
<td>6.8 ± 1.0*</td>
<td>7.4 ± 0.8</td>
<td>6.1 ± 0.7</td>
</tr>
</tbody>
</table>

*p < 0.05 compared to control; number of animals are given in parentheses.

**Discussion**

Our previous study showed that CWE of *S. acmella* flowers possesses antinociceptive activity against phasic pain (Pieris et al., 2001). The results of this study demonstrate that CWE of *S. acmella* flowers has oral antinociceptive activity against acute continuous inflammatory pain (as evaluated by the formalin test) and also possesses antihyperalgesic activity (as judged by carrageenan-induced thermal hyperalgesia). Both these effects are real and not due to generalized muscle impairments, as CWE of *S. acmella* flowers are reported to be incapable of curtailing the reaction times in the bar holding and Bridge tests (Plaznic et al., 1993) and because it failed to induce hypothermia in this study. This is a novel and an important finding: continuous inflammatory pain is one of the most common types of pathological pain in clinical practice (Crook et al., 1984) and persistent pain is known to have a major impact on the quality of life (Crook et al., 1984).

In the formalin test, CWE of *S. acmella* reduced in a dose-related fashion the number of paw lickings and time spent on paw licking at both initial and late phases in more or less equal manner. The inverse dose-response relationship is probably due to reduction of the effective principle at its high concentrations as claimed for analgesic activity of mature fresh leaves of *Fitex negundo*.

**Table 3.** Prostaglandin synthesis inhibition activity of different concentrations of cold-water extract (CWE) of *S. acmella* flowers as indicated by the reduction of spontaneous contractions of isolated rat distenss uterus with respect to normal concentrations (means ± SEM).

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>% reduction in amplitude</th>
<th>% reduction in frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>27.8 ± 0.2</td>
<td>42.7 ± 0.2</td>
</tr>
<tr>
<td>10</td>
<td>28.4 ± 3.9</td>
<td>45.4 ± 0.5</td>
</tr>
<tr>
<td>30</td>
<td>25.5 ± 0.5</td>
<td>49.9 ± 0.2</td>
</tr>
</tbody>
</table>

Significant (p < 0.05) relationship between concentration and % inhibition was evident (linear regression analysis).

**Table 4.** Antihistamine activity of cold-water extract (CWE) of *S. acmella* flowers as determined by histamine-induced wheal test in rats (means ± SEM).

<table>
<thead>
<tr>
<th></th>
<th>Area of the wheal (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>99.0 ± 7.22</td>
</tr>
<tr>
<td>Chlorpheniramine (n = 6)</td>
<td>52.2 ± 4.09*</td>
</tr>
<tr>
<td>1500 mg/kg CWE (n = 6)</td>
<td>74.6 ± 7.36*</td>
</tr>
</tbody>
</table>

*p < 0.05 as compared to control; number of animals used is given in parentheses.
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(Dharmasiri et al., 2003) and or due to presence of an antagonistic constituent(s) at high concentrations. Impairment of these two nociceptive parameters in the initial phase suggests that CWE of the flowers may interrupt nociception transmission of sensory C-fibers (Tjolsen et al., 1992). However, this modulation of nociceptive transmission is unlikely to be due to a membrane stabilizing effect of the CWE, as the CWE failed to induce a change in the absorbance of the heat-induced hemolysis of rat erythrocytes in vitro. Membrane stabilizing agents decrease the absorbance in this test (Dharmasiri et al., 2003). It is possible that the CWE may be modulating nociceptive signals in the afferent C-fibers by acting on ligand-gated ion channels that respond to chemical stimulation (Walker et al., 2003) to impair the first phase of the formalin test. Additional experiments are needed to clarify this point. Opioids also inhibit the first phase of the formalin test (Tjolsen et al., 1992). However, opioidomimetic mode of action is unlikely as naloxone, an opioid receptor antagonist, has failed to block antinociception induced by CWE of S. acmella flowers in the hot-plate test (Pieris et al., 2001).

The pain induced in the late phase of the formalin test is due to release of inflammatory mediators such as prostaglandins (histamine, bradykinin) at the site of the injection (Tjolsen et al., 1992). The antinociceptive effect that was evident in this phase of the formalin test may be due to prostaglandin synthesis inhibitory activity of CWE, as it impaired the frequency of spontaneous contractions of the isolated rat diestrous uterine preparation (Dharmasiri et al., 2003) and because prostaglandin synthesis inhibitors are potent analgesics (Rang et al., 1995). CWE of the flowers also exhibited strong antihistamine activity as revealed by the histamine wheal test (Spector, 1956). Such an action could inhibit the activity of histamines released due to injury of the nerve resulting from formalin injection. Opioids can also inhibit the late phase of the formalin test (Tjolsen et al., 1992) but such a mode of action is unlikely as explained earlier.

The carrageenan-induced thermal hyperalgesia test revealed that the CWE has strong antihyperalgesic activity. This effect was dose-related. Formalin is now claimed to develop a hyperalgesic state in the late phase of the formalin test (Kaufmann et al., 1997). Suppression of pain induced in the second phase of this test by the CWE may provide additional evidence for antihyperalgesic activity. Further, the onset of the antihyperalgesic effect was extremely rapid (within ½h) and had a fairly long duration of action (up to 6h). However, the antihyperalgesic potential of the CWE was lower than that of morphine, especially in the initial stages.

Because carrageenan induces hyperalgesia by both peripheral and central mechanisms (Richardson et al., 1998), CWE's antihyperalgesic activity could be at either site. Prostaglandin synthesis inhibiting activity and antihistamine activity are two possible mechanisms of CWE-induced antihyperalgesia. CWE of S. acmella flowers is reported to have sedative activity (Pieris et al., 2001). Sedatives have analgesic activity (Rang et al., 1995), and this mode of action may also contribute to its antihyperalgesia. Interruption of nociception transmission along C-fibers (as explained previously) may also play a vital role in inducing antihyperalgesia. Membrane stabilizers can induce antihyperalgesia (Rang et al., 1995), but the CWE did not have such activity. There is evidence that free radicals contribute to the development of hyperalgesia (Halliwell, 1994). However, the CWE did not possess antioxidant activity in vitro, and such a mode of antihyperalgesic action seems unlikely.

Phytochemically, flowers of S. acmella are reported to contain amino acids (Pieris et al., 2001), alkaloids, and N-isobutylamides (spilanthol, undeca-2E,7Z,9E-trienoic acid isobutylamide, and undeca-2E-en-8,10 diynonic acid isobutylomide (Ramsewak et al., 1999). The antinociceptive against acute inflammatory pain and the antihyperalgesic activity of S. acmella flowers in this study is likely to be mediated via one or more of these constituents.

In a toxicity study, CWE of S. acmella flowers has been shown to be well tolerated (in terms of overt signs, serum GOT, GPT, creatinine, and urea levels) even after subchronic treatment at high dose levels (Pieris et al., 1991; Ratnasooriya et al., 2004). This is a positive point for potential therapeutic use of S. acmella flowers as an antinociceptive and antihyperalgesic drug.

In conclusion, this study shows for the first time, by scientifically controlled experimentation, that CWE of S. acmella flowers has both antinociception activity against persistent inflammatory pain and antihyperalgesic activity.

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