Anti-inflammatory activity of roots of Achyranthes aspera

S. Vijaya Kumar1, P. Sankar2, and R. Varatharajan3

1School of Pharmacy, INTI International University College, Persiaran Perdana BBN, Putra Nilai, Nilai, Negeri Sembilan, Malaysia, 2Department of Pharmaceutical Chemistry, K.M. College of Pharmacy, Uthangudi, Madurai, Tamil Nadu, India, and 3School of Pharmacy, Masterskill College of Nursing & Health, Jalan Kemachaya, Cheras, Selangor Darul Ehsan, Malaysia

Abstract
This study investigated the anti-inflammatory potential of the alcohol extract of Achyranthes aspera Linn. (Amaranthaceae) in Wistar rats after oral administration (50, 100, and 200 mg/kg). This was done using the carrageenan-induced paw edema method (acute inflammatory model) and cotton pellet granuloma test (chronic inflammatory model). The alcohol extract showed significant suppressed granuloma formation. Collectively, these data demonstrate promising anti-inflammatory activity against both acute and chronic inflammation. In addition, inhibition of prostaglandins and bradykinins may play a role. This study revealing the promising anti-inflammatory activity of Achyranthes aspera roots has been carried out scientifically for the first time.

Keywords: Alcohol extract; Achyranthes aspera (Amaranthaceae); anti-inflammatory activity; roots

Introduction
The search for new pharmacologically active agents obtained by screening natural sources such as microbial fermentations and plant extracts has led to the discovery of many clinically useful drugs that play a major role in the treatment of human diseases (Hostettmann, 1997). In India, a small proportion of wild plants have been investigated both phytochemically and pharmacologically. Achyranthes aspera Linn. (Amaranthaceae) is a wild tropical plant. The isolated achyranthine (Kapoor, 1996) is used in traditional medicine to treat many ailments and is also recommended for the treatment of menstrual disorder (Bhatterjee, 2001). The leaves of Achyranthes aspera are used in the treatment of dermatological disorders (Jayaweera, 1982). Further, a decoction of flowers and barks is given for hemoptysis and dysmenorrhea. We report here on the anti-inflammatory activity of the alcohol extract of Achyranthes aspera using the carrageenan-induced paw edema method (acute inflammatory model) and cotton pellet granuloma technique (chronic inflammatory model) in Wistar rats.

Materials and methods

Animals
Healthy adult cross-breed albino male Wistar rats (150–200 g) were used in the study. The animals were kept in plastic cages (six per cage) under standardized animal house conditions (temperature, 28–31°C; photoperiod, approximately 12 h natural light “per day”; relative humidity, 50–55%) with continuous access to pellet feed and tap water. Every effort was made to minimize animal suffering and to reduce the number of animals used in this study. The ”Principles of Laboratory Animal Care“(NIH publication no: 85-23) guidelines and procedures were also used in this study (National Institutes of Health, 1985).
Plant material

*Achyranthes aspera* was collected from fields of the Agricultural Research Station, Sivaganga, Tamil Nadu, India in March 2005 and identified and authenticated by Professor B. Ananthanarayanan, Department of Botany, Madurai Kamaraj University. A voucher specimen (DBH) was deposited at the Museum of the Department of Zoology.

Extraction and isolation

The air-dried root (148 g) was powdered and extracted with ethyl alcohol (50–60ºC) for 18 h using the Soxhlet extraction process. The extract was subjected to vacuum distillation under reduced pressure, and a yellowish brown residue (12.6 g, 5.6%) was obtained. The alcohol extract was then chromatographed over a column of silica gel and eluted with chloroform to give a colorless compound, identified by positive sterol color test with the Libermann reaction and thin layer chromatography (TLC) on an authentic sample.

Anti-inflammatory activity

Carrageenan-induced paw edema

Forty male Wistar rats were selected and randomly divided into five groups. The Wistar rats in groups 1, 2, and 3 were orally treated with 50, 100, and 200 mg/kg of aqueous alcohol extract respectively. The Wistar rats of the fourth group were treated with 1 mL of distilled water. The Wistar rats of the fifth group were treated with 50, 100, and 200 mg/kg of the reference drug indomethacin (Laurence & Bennett, 1992). One hour after administration of extract, 0.05 mL of 1% carrageenan (Sigma Chemical Co., St. Louis) suspension was injected subcutaneously into the plantar surface of the left hindpaw (Winter et al., 1962). The volumes of Wistar Rat hindpaws in the test, control, and standard groups were measured using a plethysmometer (Letica Scientific Instruments, Barcelona, Spain) at 1, 2, 3, 5, and 8 h after the induction of inflammation, and edema was expressed as an increase in paw volume due to carrageenan injection.

Cotton pellet granuloma

Twelve Wistar rats were randomly assigned into two groups (each group containing six rats). Autoclaved cotton pellets (10 mg) were implanted subcutaneously, one on each side above the scapula, under ether anesthesia using aseptic precautions (Dhawan & Srimal, 2000). Either 200 mg/kg of alcohol extract of *Achyranthes aspera* or 1 mL of distilled water was administered orally for 7 days starting from the day of injection. On the 8th day the animals were sacrificed, and the pellets along with granulomas were removed and dried in an oven at 60ºC until a constant weight was obtained.

Statistical analysis

The data are expressed as the mean ± SD. Statistical analysis was performed using Dunnett’s *t*-test. *p* ≤ 0.05 was considered statistically significant.

Results

Carrageenan-induced paw edema

The results obtained are summarized in Table 1. As shown, all the doses of alcohol extract of *Achyranthes aspera* tested caused a significant (*p* < 0.05) and marked reduction in paw edema (32–40.5%) compared to control at each time point measured. Indomethacin also impaired the edema formation, but this anti-inflammatory effect was much stronger (32–40%).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)×</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>5 h</th>
<th>8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>39.8 ± 5.6</td>
<td>54.4 ± 6.8</td>
<td>65.5 ± 4.7</td>
<td>68.4 ± 4.1</td>
<td>58.4 ± 2.1</td>
</tr>
<tr>
<td>Alcohol extract</td>
<td>50</td>
<td>27.4 ± 2.9* (32.0)</td>
<td>42.0 ± 1.2* (26.7)</td>
<td>48.5 ± 4.2* (25.0)</td>
<td>36.5 ± 1.9* (44.0)</td>
<td>42.5 ± 2.8* (28.5)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>32.4 ± 3.1* (28.5)</td>
<td>51.1 ± 3.2* (19.5)</td>
<td>60.4 ± 2.6** (23.0)</td>
<td>45.4 ± 1.6** (38.0)</td>
<td>48.2 ± 1.8* (25.0)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>25.0 ± 2.5** (38.6)</td>
<td>44.2 ± 2.5* (28.5)</td>
<td>51.2 ± 3.9* (26.4)</td>
<td>38.1 ± 2.0* (40.5)</td>
<td>35.0 ± 2.0* (32.5)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>50</td>
<td>14.5 ± 3.2* (63.0)</td>
<td>17.3 ± 1.4* (68.0)</td>
<td>21.5 ± 8.4* (65.1)</td>
<td>35.8 ± 4.3* (43.5)</td>
<td>30.1 ± 4.1** (48.0)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>17.5 ± 4.0* (58.5)</td>
<td>22.0 ± 6.2* (60.0)</td>
<td>36.2 ± 9.0* (43.2)</td>
<td>54.5 ± 3.8* (26.0)</td>
<td>38.4 ± 3.5* (45.0)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>13.0 ± 3.2* (65.0)</td>
<td>21.0 ± 5.4* (65.2)</td>
<td>33.5 ± 6.8* (48.0)</td>
<td>56.4 ± 4.2* (16.0)</td>
<td>32.5 ± 2.8* (46.0)</td>
</tr>
</tbody>
</table>

×mg/kg body weight; values represent mean ± SD of six animals for each group.

*Each value in parentheses indicates the percentage inhibition rate.

*p* ≤ 0.01 and **p** ≤ 0.05, statistically significant from control (Dunnett’s *t*-test).
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Cotton pellet granuloma

The alcohol extract of Achyranthes aspera caused a significant ($p < 0.05$) and marked inhibition (by 34.6%) of granuloma weight as compared to control (control vs. test: 29.6 ± 9.4 vs. 19.0 ± 7.2 mg).

Discussion

The alcohol extract of Achyranthes aspera was evaluated for anti-inflammatory activity in Wistar rats using the carrageenan-induced paw edema test (acute inflammatory model) and cotton pellet test (chronic inflammatory model) after oral administration. The results show that the alcohol extract of Achyranthes aspera has promising anti-inflammatory activity both against acute (exudative phase) and chronic (proliferative phase) inflammation. Maximum inhibition of paw edema has previously been reported (Gupta et al., 2000).

According to Vineagar et al. (1987), the development of paw edema is derived from the release of cytoplasmic enzymes and serotonin from mast cells and the increase of prostaglandin in the inflammatory area. The macrophages in carrageenan-insulted dermal tissue release interleukin-1 causing accumulation of polymorphic nuclear cells (PMNs) in the inflammatory area; the activated PMNs then release lysosomal enzymes and active oxygen, which induces paw swelling.

To conclude, this study revealing the promising anti-inflammatory activity of Achyranthes aspera roots has been carried out scientifically for the first time. The evaluation could be an important finding globally, as inflammation has become a common disease condition with “poor availability of drug therapies” (Anonymous, 1976). About four billion people worldwide rely on plants as sources of drugs (Farnsworth, 1988), and in India around 40% of the population depend on traditional medicine for their primary healthcare needs (Mahindapala, 2000). Roots of Achyranthes aspera are noncommercial and abundantly available throughout the year.

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References
