EFFECT OF ALCOHOLIC EXTRACT OF ACHYRANTHES BIDENTATA BLUME ON ACUTE AND SUB ACUTE INFLAMMATION

T. VETRICHELVAN, M. JEGADEESAN*

Adhiparasakthi College of Pharmacy, Melmaruvathur-603 319.
*Department of Siddha Medicine, Faculty of Sciences, Tamil University, Thanjavur-613 005.

Manuscript Received: 21.6.2001        Revised: 3.10.2001    Accepted: 25.10.2001

ABSTRACT

Objective: To study the anti-inflammatory activity of alcoholic extract of Achyranthes bidentata on carrageenan-induced hind paw oedema and cotton pellet granuloma models in Swiss male rats.

Methods: The hind paw oedema was produced by subplantar injection of carrageenan and the paw volume was measured plethysmographically at 0, 1, 2, 3, 4 and 5 h. In sub-acute model, cotton pellet granuloma was produced by implantation of 50±1 mg sterile cotton in axilla under ether anaesthesia. The animals were fed with ethanolic extract at various dose levels (125, 250, 375 and 500 mg/kg). Diclofenac sodium was used as a standard drug.

Results: The alcoholic extract (375 and 500 mg/kg) showed maximum inhibition of oedema by 63.52% and 79.73% at the end of 3 h in acute model of inflammation, respectively. Using a chronic test, the granuloma pouch in rats, the extract exhibited a 50.76% and 57.49% reduction in granuloma weight.

Conclusion: Achyranthes bidentata possesses anti-inflammatory effects in both acute and sub acute inflammation.

KEYWORDS
Achyranthes bidentata    anti-inflammatory activity    diclofenac sodium    paw oedema

INTRODUCTION

Achyranthes bidentata (Amaranthaceae; Tamil name-Sigappu Nayurivi) is an erect, annual herb distributed in hilly districts of India, Java, China and Japan. The plant is used in indigenous system of medicine as emenagogue, antiarthritic, antifertility, laxative, eczobic, abortifacient, anthelmintic, aphrodisiac, antiviral, antispasmodic, antihypertensive, anticoagulant, diuretic and anti-tumour. Also it is useful to treat cough, renal dropsy, fistula, scrofula, skin rash, nasal infection, chronic malaria, impotence, fever, asthma, amennorrhoea, piles, abdominal cramps and snake bites. The phytochemical studies revealed that it contains rutin, saponins, achyranthine, caffeic acid, oleanolic acid, inokosterone, ecdysterone, rubrosterone and physcion. A. bidentata have been reported to have antirheumatic activity in folklore practice (personal communication). To substantiate this claim, the present study was undertaken to evaluate the anti-inflammatory potential of this plant extract on carrageenan-induced rat hind paw oedema and in the granuloma test in rats.

MATERIALS AND METHODS

Preparation of extract: Whole parts of A. bidentata was collected from the hilly region of Acharapakkam, Kanchipuram District, Tamilnadu, India. The whole plant material was reduced to small pieces, dried under shade, powdered in a pulveriser and passed through a 80 mesh sieve. The powdered plant was packed into a Soxhlet apparatus (350 g) and dewaxed with benzene. The dried dewaxed powder was extracted sequentially with 50% ethanol. After completion of extraction, filtered and the solvent was removed by distillation under reduced pressure. The dried benzene (1.96% W/W) and alcoholic (16.01% W/W) extracts were subjected to qualitative chemical tests for the detection of phytoconstituents.
preliminary phytochemical studies showed the presence of flavonoid glycosides in alcoholic extract. The alcoholic extract was suspended in 0.75 % carboxy methyl cellulose (CMC) and employed for evaluation of anti-inflammatory activity.

**Animals:** Swiss male rats (B.W. 140-170 g) were used. They were housed in standard microlon boxes and were given standard laboratory diet and water *ad libitum*.

**Carrageenan-induced paw oedema:** The rats were divided into six groups (n=8) and the first group served as negative control (received 0.75% CMC; 5 ml/kg). Second group was administered diclofenac sodium (5 mg/kg) as a standard drug. Group 3 to 6 were fed with alcoholic extract (125, 250, 375 and 500 mg/kg)\(^8\) orally. Edema was produced by the method described by Winter *et al* (1962)\(^9\). The paw volume was measured plethysmographically at 0, 1, 2, 3, 4 and 5 h, after the injection of carrageenan. Drug-pretreatment was given 1 h before the injection of carrageenan. Mean increase in paw volume was measured and percentage inhibition was calculated.

**Cotton pellet granuloma:** Sub-acute inflammation was produced by the method described by Winter *et al* (1957)\(^10\). Sterile cotton (50 ± 1mg) soaked in 0.2 ml of distilled water containing penicillin (0.1 mg) and streptomycin (0.13 mg) was implanted subcutaneously bilaterally in axilla under ether anaesthesia. Animals were divided into six groups (n=6). Extract (125, 250, 375 and 500 mg/kg), diclofenac sodium and control vehicle were administered daily for 10 days (0 to 9 days). On the 10th day the pellets were dissected out, dried at 60° C, and the dry weight was determined. The weight of the cotton pellet before implantation is subtracted from the weight of the dried, granuloma pellets. The results were expressed as mean ± SEM. The differences were compared using one-way ANOVA followed by Dunnett’s test. P values < 0.05 were considered significant.

**Table 1.** Effect of alcoholic extract of *A. bidentata* on carrageenan-induced rat paw oedema.

<table>
<thead>
<tr>
<th>Group (n=8)</th>
<th>Dose mg/kg</th>
<th>Oedema volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>Control (0.75 % CMC)</td>
<td>5 ml</td>
<td>0.62 ± 0.05</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>5</td>
<td>0.24 ± 0.03(^b) (61.30)</td>
</tr>
<tr>
<td>Alcoholic extract</td>
<td>125</td>
<td>0.51 ± 0.04(^b\NS) (17.75)</td>
</tr>
<tr>
<td>Alcoholic extract</td>
<td>250</td>
<td>0.49 ± 0.04(^b\NS) (21.96)</td>
</tr>
<tr>
<td>Alcoholic extract</td>
<td>375</td>
<td>0.42 ± 0.04(^a) (32.26)</td>
</tr>
<tr>
<td>Alcoholic extract</td>
<td>500</td>
<td>0.35 ± 0.05(^a) (43.55)</td>
</tr>
</tbody>
</table>

One-way ANOVA

- p<0.001; \(^b\)p<0.01; \(^c\)p<0.001 compared to control.
- NS: Statistically not significant; degrees of freedom (5, 42).

Each value is the mean ± SEM of 8 rats.

Figures in parentheses indicate the % anti-inflammatory activity.
RESULTS

In acute inflammation model, the extract showed maximum inhibition of the carrageenan-induced rat paw oedema at the end of 3 h (Table 1). Edema suppressant effect of 375 and 500 mg/kg treated groups were found to be significant (p<0.001) as compared to control. Diclofenac showed similar type of reduction (p<0.001) as compared to the control rats. Also the alcoholic extract of A. bidentata produced a dose-dependent inhibition of carrageenan-induced rat hind paw oedema.

In sub-acute inflammation model, the weight of the granulation tissue formation was significantly (p<0.001) reduced by the extract (375 and 500 mg/kg) and diclofenac sodium. The extract also showed dose-dependent inhibitory effect on granuloma weight. The percentage inhibition of the plant extract (500 mg/kg) was found to be almost similar to that of 5 mg/kg of diclofenac sodium (Table 2).

DISCUSSION

The probable mechanism of action of carrageenan-induced oedema is bi-phasic, the first phase is attributed to the release of histamine, 5-HT and kinins in the first hour; while, the second phase is related to the release of prostaglandin like substances in 2-3 h. Effect of alcoholic extract at a dose of 375 and 500 mg/kg are dose dependent and significant in inhibiting carrageenan-induced oedema. On preliminary phytochemical screening, the alcoholic extract showed the presence of alkaloids, terpenes, amino acids, flavonoids and its glycosides. Characterization of the flavonoid structure in alcoholic extract has been elucidated as quercetin and its rutinoside by Nguyen et al (1995). Quercetin is a flavonoid effective in acute inflammation, whereas its rutinoside is effective in sub-acute inflammation. Hence, the significant anti-inflammatory activity of A. bidentata could be due to the presence of a flavonoid, which may exert predominant inhibition of inflammatory mediators from phlogogenic stimuli. Alcoholic extract of A. bidentata has shown potential inhibitory action on exudate formation. Kinin is said to be main mediator of granuloma, as it both vasodilates and increases vascular permeability in the early stages of inflammation. Sub-acute inflammation involves infiltration of macrophages, neutrophils and proliferation of fibroblasts. Hence, the decrease in granuloma weight indicated the anti-proliferative activity of flavonoids in alcoholic extract of A. bidentata. The present study has further encouraged the folklore practice of A. bidentata in the treatment of inflammation associated diseases like arthritis.

ACKNOWLEDGEMENTS

The authors are grateful to Arulthiru Bangaru Adigalar, President and Thirumathi Lakshmi Bangaru Adigalar, Vice-President, Adhiparasakthi, Charitable, Medical, Educational and Cultural Trust, Melmaruvathur, for providing all facilities to carry out this research work. Also we are thankful to Dr. S.K. Kaskhedikar, Department of Pharmacy, SGSITS, Indore, for valuable suggestions.

REFERENCES

NEW TARGET FOR ANTIMALARIAL DRUGS

An international team of researchers report that they have identified a potential new class of drugs for the treatment of malaria. The development of these new antimalarial drugs for oral administration as both preventive and curative malaria treatment in humans is now a realistic objective. Team leader Henri Vial (Université Montpellier II and CNRS, France) has announced that the investigators now aim to develop an oral formulation that can be used as a cheap alternative to chloroquine.

During asexual multiplication of the malaria parasite within erythrocytes, large amounts of membrane are synthesised, for which phospholipid biosynthesis is essential. The biosynthesis of phosphatidylcholine in Plasmodium is of particular interest since it is the most abundant lipid, accounting for half of the total phospholipid in parasite membranes, and the most promising drug interference is blockage of the choline transporter that provides the intracellular parasite with choline, a precursor required for synthesis of this major parasite phospholipid.

Vial and colleagues have tested the effects of G25, a choline analogue that binds competitively to the choline carrier, thus inhibiting choline transport into infected erythrocytes. They found that intramuscular injection of G25 completely cured monkeys that were infected with either \( P \) falciparum or \( P \) cynomolgi malaria. G25 was effective at doses far below those used for current antimalarial drugs, and there was no recrudescence of disease. Oral administration of G25 also appeared to be fully effective. The researchers are now concentrating their efforts on developing new compounds that have improved oral absorption which is essential for dispensaries in endemic countries that often do not have adequate facilities to safely give drug injections. Preclinical studies with a suitable drug are likely to begin in the next two years.