

Preliminary Studies of Analgesic and Anti-inflammatory Properties of *Antigonon leptopus* Hook. et Arn Roots in Experimental Models

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The methanolic extract of *Antigonon leptopus* Hook. et Arn roots was evaluated for analgesic and anti-inflammatory activity at the doses of 200 and 400 mg/kg body weight. The hot-plate and acetic acid writhing response in mice were used to assess analgesic activity. Carrageenan-induced paw edema in rats, which is an acute model, was used to evaluate the anti-inflammatory activity of *A. leptopus* Hook. et Arn root extract. The extract inhibited paw edema in a dose-related manner. A dose-dependent analgesic action was obtained against chemical (writhing test) and thermic (hot-plate test) stimuli indicating antinociceptive activity may involve inhibition of pain by peripheral and central mechanisms. Further, an acute toxicity study with the extract showed no sign of toxicity up to a dose level of 2000 mg/kg body weight. Thus, the extract of *A. leptopus* Hook. et Arn root possesses significant analgesic and anti-inflammatory properties.

Key words — *Antigonon leptopus*, anti-inflammatory, analgesic, writhing, nociception

INTRODUCTION

Antigonon leptopus Hook. et Arn (syn: *Corculum leptopus* family: Polygonaceae) or coral vine is grown in parks and gardens throughout India. It is most common in the upper Ganges plains and Himalayan regions. It is a fast growing climber with heart shaped green leaves, flowers through summer to autumn with coral pink to red flowers hanging in panicles up to 15 cm long, and will climb up to 40 ft to protect itself from frost. Traditionally the leaves have been used to reduce swelling, and a tea from the leaves can be made to treat diabetes and from the blossoms to treat high blood pressure. The vine is used to treat cough and throat constriction.¹⁾ It has anticoagulant activity.²⁾ Information gathered from local herbal healers where the plant was collected revealed that the roots of the plant are useful to reduce pain and inflammation. The purpose of the present study was to evaluate the possible

antinociceptive activity of a methanolic extract of *A. leptopus* Hook. et Arn root (MEAL) by using the writhing test, hot-plate test, and anti-inflammatory effect by using the carrageenan-induced edema test, which is an acute model.

MATERIALS AND METHODS

Plant Material — The roots of *A. leptopus* Hook. et Arn were collected in April 2006 from Visakhapatnam, Andhra Pradesh. The plant material was authenticated at Dept. of Botany, Kakatiya University, Warangal. The voucher specimen (KU/UCPSc/15/2006) of this plant material has been retained in the Dept. of Pharmacognosy and Ethnopharmacology, University College of Pharmaceutical Sciences, Warangal.

Preparation of Extract — The roots were cut into small pieces and shade dried and then ground into coarse powder for the maceration process with methanol at room temperature. After exhaustive extraction, the methanolic extract was concentrated under reduced pressure at 50–55°C and stored in a vacuum desiccator. A fine suspension of the extract

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prepared in 2% gum acacia was used for the experiments.

Animals — Wistar rats (150–250 g) and albino mice (20–25 g) of either sex were used. Animals were maintained under standard environmental conditions and had free access to feed and water *ad libitum*. The animals were acclimatized to the laboratory environment for at least one week before the experimental session and then divided groups consisting of 6 animals. For experimentation, the animals were fasted overnight. Experiments on animals were performed in accordance with the guidelines of the Institutional Animal Ethics Committee.

Antinociceptive Tests —

Writhing Test: Abdominal constriction induced by intraperitoneal injection of acetic acid was carried out according to the procedures described previously.³⁾ *A. leptopus* root extract was tested at 200 and 400 mg/kg. Diclofenac sodium, a reference anti-inflammatory and analgesic compound, was used at 20 mg/kg. The extract and reference drug were administered orally 30 min before the administration of 0.7% acetic acid in a volume of 10 mg/kg intraperitoneally. Control animals received 2% gum acacia under the same experimental conditions. Immediately after injection of the acetic acid, each animal was isolated in an individual cage and the number of constrictions was cumulatively counted for a period of 20 min, beginning 3 min after acetic acid injection. The number of writhings and stretchings was recorded and the percentage was calculated using the following ratio:

$$\text{Percentage of protection} = \frac{\text{Control mean} - \text{Treated mean}}{\text{Control mean}} \times 100$$

Hot-plate Test: In the hot plate test, mice were placed on an enclosed copper hot-plate maintained at $55 \pm 0.5^\circ\text{C}$ and the time between placement of the mice on the hot-plate and the occurrence of either a hind paw lick or a jump off the surface was recorded as the hot-plate latency. Mice with baseline latencies of > 20 sec were eliminated from the study. After determination of baseline response latencies, *A. leptopus* root extract was tested at 200 and 400 mg/kg and the hot-plate latencies were determined at 0, 60, 120 and 240 min. Pentazocine 10 mg/kg i.p., was used as the reference drug. For each group, the average reaction times were the fol-

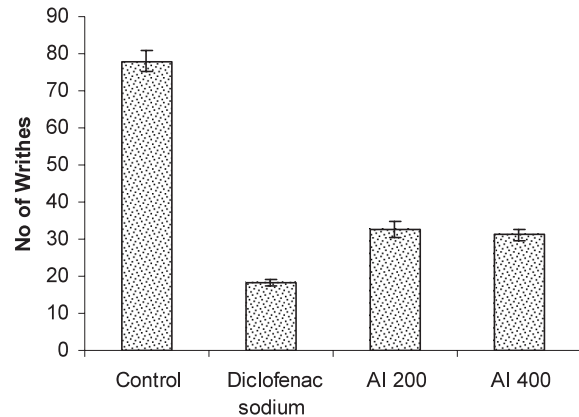


Fig. 1. Effect of *A. leptopus* Extract on Acetic Acid-induced Writhing Response in Mice

The data are expressed as mean \pm SEM of 6 animals. AI 200, *A. leptopus* 200 mg/kg; AI 400, *A. leptopus* 400 mg/kg; * $p < 0.05$, ** $p < 0.01$ as compared with control.

lowing ratio:

$$\text{Percentage variations} = \frac{\text{Drug latency} - \text{Baseline latency}}{\text{Baseline latency}} \times 100$$

Anti-inflammatory Test —

Carrageenan-induced Rat Paw Edema: To study the effect of *A. leptopus* Hook. et Arn root extract on acute inflammation, the carrageenan-induced rat paw edema model was used.⁴⁾ Carrageenan (0.1 ml of a 1% suspension in saline) was injected subplantarily into the midline midmetatarsal region of the right hind paw of the rat. Thirty minutes prior to the sterile injection of the phlogistic challenge, the extract (200 and 400 mg/kg) was administered orally. Diclofenac sodium 20 mg/kg was used as the reference anti-inflammatory drug. Control animals received 2% gum acacia under the same experimental conditions. The extract, diclofenac sodium, and gum acacia were administered orally in a volume of 0.5 ml/kg of body weight. Each treated group was composed of six rats. The volumes of the injected and the control paws were measured 1, 2, 3, and 4 hr after induction of inflammation.

These individual records allowed determination of the volumes for each group (V_t) and then the percentages of edema, by comparison volume obtained for each group before any treatment (V_0), using the following ratio:

$$\text{Percentage edema} = \frac{V_t - V_0}{V_0}$$

The percentages of inhibition were obtained for each group and at each record, using the following

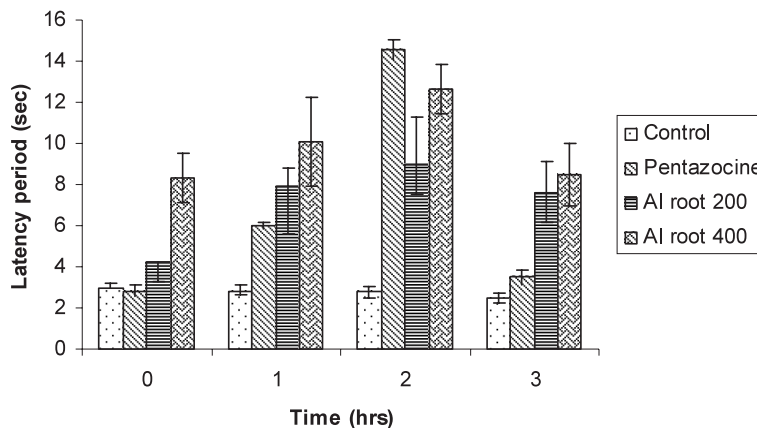


Fig. 2. Effect of *A. leptopus* on Mice Exposed to a Hot-plate

The data are expressed as the mean ± SEM of 6 animals. Al 200, *A. leptopus* 200 mg/kg; Al 400, *A. leptopus* 400 mg/kg; **p* < 0.05, ***p* < 0.01 as compared with control.

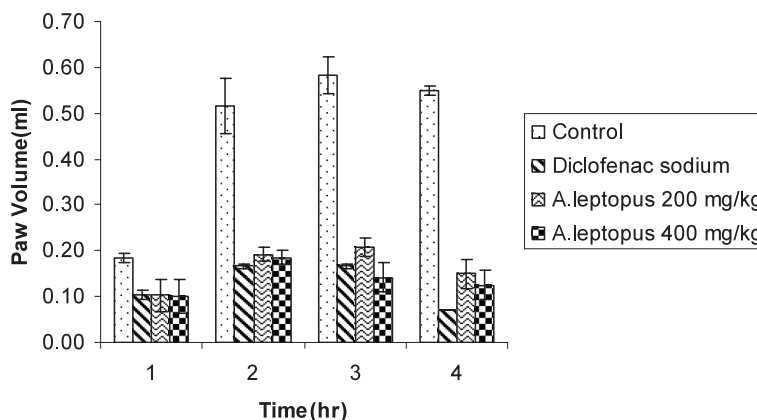


Fig. 3. Effect of *A. leptopus* on Rat Hind Paw Edema Induced by Carrageenan

The data are expressed as the mean ± SEM (*n* = 6). **p* < 0.05, ***p* < 0.01 as compared with control.

ratio:

$$\text{Percentage inhibition} = \frac{(V_o - V_t) \text{ Control} - (V_t - V_o) \text{ Treated}}{(V_o - V_t) \text{ Control}} \times 100$$

Drugs— Carrageenan (SD Fine Chemicals Limited, Mumbai, India), gum acacia (Hi-media, Mumbai, India), diclofenac sodium (Dr. Reddy Labs, Hyderabad, India), pentazocin (Pure Pharma Ltd., Mumbai, India), methanol (BDH, Mumbai, India) and acetic acid (Ranbaxy Laboratories Ltd., Punjab, India).

Statistical Analysis— The values are expressed as the mean ± SEM and were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s *t*-test. *p*-values < 0.05 were considered as significant. All statistical manipulations were car-

ried out using Graph Pad Prism 3.0 (GraphPad Software, Inc., U.S.A.) statistical software.

RESULTS

Phytochemical Screening

Preliminary phytochemical screening of the methanol extract revealed the presence of steroids, flavonoids, tannins, alkaloids and glycosides.

Acute Toxicity Study

In the acute toxicity study, no mortality was observed during the 24 hr period at the doses tested and the animals showed no stereotypical symptoms associated with toxicity, such as convulsions, ataxia, diarrhea or increased diuresis.

Table 1. Analgesic Effect of the Methanolic Extract of *A. leptopus* Hook. et Arn Root on Acetic Acid-induced Nociception

S.No	Group	Dose (mg/kg)	No of writhings (mean \pm SEM)	Inhibition (%)
1.	Control		78.00 \pm 2.78	—
2.	Diclofenac sodium	20	18.33 \pm 0.95**	76.50
3.	<i>A. leptopus</i>	200	32.60 \pm 2.28**	58.20
4.	<i>A. leptopus</i>	400	31.16 \pm 1.66**	60.04

Values are mean \pm SEM; $n = 6$; significance at * $p < 0.05$, ** $p < 0.01$ as compared to the control.

Table 2. Analgesic Effect of the Methanolic Extract of *A. leptopus* Hook. et Arn Root Using the Hot-plate Method

S.No	Group	Dose (mg/kg)	Reaction time after administration of control/standard/extract in sec			
			0	60	120	240
1.	Control		3.00 \pm	2.83 \pm	2.78 \pm	2.50 \pm
			0.22	0.27	0.28	0.22
2.	Pentazocine	10	2.83 \pm	6.00 \pm	14.58 \pm	3.50 \pm
			0.27	0.18	0.47**	0.38
3.	<i>A. leptopus</i>	200	4.27 \pm	7.89 \pm	9.0 \pm	7.63 \pm
			0.95	2.30**	1.48**	1.43**
4.	<i>A. leptopus</i>	400	8.33 \pm	10.1 \pm	12.66 \pm	8.50 \pm
			1.2	2.14**	1.20**	1.52**

Values are mean \pm SEM; $n = 6$; significance at * $p < 0.05$, ** $p < 0.01$ as compared to the control.

Table 3. Anti-inflammatory Effect of the Methanolic Extract of *A. leptopus* Hook. et Arn Root on Carrageenan Induced Paw Edema

S.No	Group	Dose (mg/kg)	Paw Edema Volume			
			1 hr	2 hr	3 hr	4 hr
			Mean \pm %PEI SEM	Mean \pm %PEI SEM	Mean \pm %PEI SEM	Mean \pm %PEI SEM
1.	Control		0.18 \pm —	0.52 \pm —	0.58 \pm —	0.55 \pm —
			0.01	0.06	0.04	0.01
2.	Diclofenec sodium	20	0.10 \pm 42.68	0.17 \pm 67.66	0.17 \pm 71.45	0.07 \pm 86.96
			0.01	0.01**	0.01**	0.001**
3.	<i>A. leptopus</i>	200	0.10 \pm 43.77	0.19 \pm 62.82	0.21 \pm 64.33	0.15 \pm 72.72
			0.03	0.02**	0.02**	0.03**
4.	<i>A. leptopus</i>	400	0.10 \pm 45.44	0.19 \pm 64.18	0.14 \pm 75.82	0.13 \pm 76.9
			0.04	0.02**	0.03**	0.03**

Values are mean \pm SEM; $n = 6$; significance at * $p < 0.05$, ** $p < 0.01$ as compared to the control. % PEI indicates percentage paw edema inhibition.

Effect of Plant Extract on the Acetic Acid-induced Writhing

The results of the acetic acid writhing test in mice are given in Table 1. At doses of 200 and 400 mg/kg, the MEAL inhibited the writhing responses of mice caused by the intraperitoneal administration of acetic acid. At these doses the mean numbers of the writhes was significantly lower than the control group. The maximal inhibition of the

nociceptive response was 60.04% with the dose 400 mg/kg. Diclofenac sodium exerted a significant protective effect, inducing a protection of 76.5% at a dose of 20 mg/kg shown in Fig. 1.

Effect of Plant Extract in the Hot-plate Test

The results presented in Table 2 show that the MEAL significantly increased the reaction time of mice in a dose-related manner. This dose-

dependent effect reached 86.87% with 400 mg/kg. The antinociceptive effect of *A. leptopus* Hook. et Arn root with the highest dose peaked at 120 min after injection and then slowly diminished. Pentazocine (10 mg/kg) significantly increased the reaction time of the mice. The maximal variation was obtained after 2 hr treatment as shown in Fig. 2.

Effect of Plant Extract on Carrageenan-induced Hind Paw Edema

The percentage inhibition values, calculated for each group of MEAL and diclofenac sodium, are presented in Table 3. In control animals, the sub plantar injection of carrageenan produced a local edema that increased progressively to reach a maximal intensity 4 hr after the injection of the phlogistic agent. Methanolic extract of the *A. leptopus* Hook. et Arn root showed a significant dose-dependent reduction at 200 mg/kg (72.72%) and 400 mg/kg (76.9%) and at 20 mg/kg of diclofenac sodium (86.96%) over a period of 4 hr as shown in the Fig. 3.

DISCUSSION

The potential antinociceptive effect of the methanolic extract of *A. leptopus* Hook. et Arn root was investigated. The antinociceptive evaluation methods used in the present study were chosen in order to test different nociceptive stimuli, namely cutaneous thermic (hot-plate) and chemical visceral (writhing) stimuli. The results indicate that oral administration of the *A. leptopus* Hook. et Arn root extract exhibit a significant and dose-dependent protective effect on chemical (acetic acid injection) and thermic (heat) painful stimuli at doses of 200 and 400 mg/kg, respectively. This observation indicates that MEAL has both peripheral (writhe reduction) and central (thermal reaction time prolongation) effects. Further investigations will be necessary to obtain information about the analgesic activity profile of *A. leptopus* Hook. et Arn. Pentazocine is a mixed agonist-antagonist type of opioid analgesic exhibiting its effect by acting through kappa receptors.

Carrageenan rat paw edema is a suitable method for evaluating anti-inflammatory drugs and it been frequently used to assess the anti-edematous effect of natural products.⁵⁾ The inhibitory activity shown by the extract of *A. leptopus* Hook. et Arn root (200 and 400 mg/kg) over a period of 4 hr in carrageenan-

induced paw inflammation was quite similar to that observed in the group treated with diclofenac sodium. These results indicate that the extract acts on both the initial and later phases of inflammation. The mechanism involved in the genesis of the carrageenan-induced edema can cause the release of prostaglandins and kinins, among other substances.⁶⁾ The intraperitoneal administration of acetic acid also induces the release of prostaglandins and sympathetic system mediators.⁷⁾ The anti-edematogenic mechanism of action of *A. leptopus* Hook. et Arn may also be due to prostaglandin synthesis inhibition as described for the anti-inflammatory mechanism of aspirin-like drugs.⁸⁾ Inflammatory pain results from the release of hyper analgesic mediators (*e.g.*: prostaglandins and catecholamines) which are believed to act by regulating the sensitivity of pain receptors.^{9, 10)}

Thus, the results of the study support the traditional use of *A. leptopus* Hook. et Arn root in some painful and inflammatory conditions. However, detailed chemical and biological studies on the extract are needed to isolate and characterize the biologically-active principles for exploring *A. leptopus* Hook. et Arn as an effective herbal anti-inflammatory analgesic.

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