

Anti-inflammatory and Antinociceptive Effects of the Alcoholic Extract of Indian *Polygala arvensis* in Experimental AnimalsG. SAMMAIAH^{2*}, K. THIRUPATHI¹ AND R.S. SRIVASTAVA²¹University College of Pharmaceutical Sciences, Kakatiya University, Warangal-506009, A.P., India.²Department of Pharmaceutics, Banaras Hindu University, Varanasi-221005, India.

The alcoholic extract of *Polygala arvensis* (family Polygalaceae) was screened for antinociceptive and anti-inflammatory activities in experimental animals. The extract was administered for three consecutive days. Following an oral dose of 25 - 100 mg/kg, the extract exhibited graded dose response equivalent to 16.24% - 55.43% protection in the tail flick latent test in rats. Oral doses of 50 and 100 mg/kg of the extract administered to mice effectively increased reaction time in the hot plate method by 69.55% ($p < 0.01$) and 107.13% ($p < 0.001$) respectively as well as in analgesymeter-induced mechanical pain by 28.84% ($p < 0.5$) and 55.71% ($p < 0.05$) respectively. The extract potentiated the analgesic effect of intraperitoneally administered pentazocine (10 mg/kg) and aspirin (25 mg/kg). In the carrageenan-induced paw edema test, 50 mg/kg and 100 mg/kg oral doses of the extract decreased paw volume significantly. Dose dependent anti-inflammatory activity was observed throughout the 3 h period of observation. The extract potentiated the analgesic effect of orally administered nimesulide (50 mg/kg). This study demonstrates that extracts of *P. arvensis* have significant antinociceptive and anti-inflammatory activities.

Keywords: *Polygala arvensis*, alcoholic extract, antinociceptive, anti-inflammatory**INTRODUCTION**

Polygala arvensis Linn. (family Polygalaceae) grows as a weed in most tropical countries. It is widely distributed throughout India. It is commonly known as 'nela janumu' in Telugu and 'surjavarta' in Sanskrit [1-2]. The Lambadi people of North Telangana districts of Andhra Pradesh use this plant for the treatment of pain, gastrointestinal disorders and infectious diseases. It is widely used for wound healing, as an antibacterial and an antifungal especially effective against superficial candidiasis [3-4]. There are no records of previous research work on the traditional medicinal uses of *P. arvensis*. Most of the ancient knowledge concerning the use of this plant has persisted through oral communication spanning many generations in rural communities. Preliminary

phytochemical studies revealed presence of flavonoid glycosides, flavones, flavonoids, tannins, lignans and fatty acids. The present study was undertaken to demonstrate the antinociceptive and anti-inflammatory activities of the alcoholic extract of the whole plant material of *P. arvensis* in experimental animals.

EXPERIMENTAL**Plant material**

Whole plant material of *P. arvensis* Linn. was collected around Warangal University Campus in Southern India. The plant material was identified in August 1996 and taxonomically authenticated by V.S. Raju of the Department of Botany at Kakatiya University, Warangal. A voucher specimen

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of the plant was deposited at the herbarium for future reference.

Alcoholic extraction

The alcoholic extract was prepared from a powder of the whole plant material obtained with the aid of an electric grinder. About 500 g of powder was extracted with 95% v/v alcohol using the Soxhlet apparatus. The extract was reduced under vacuum and dried in a vacuum desiccator.

Test animals

Charles-Foster (CF) albino rats and Wistar mice of either sex weighing 110-130 g and 15-19 g respectively were obtained from the animal house of the Department of Pharmaceutics, Banaras Hindu University, Varanasi, India. They were kept in the departmental animal house at 25 ± 2 °C, a relative humidity of 45.0 - 51.5% and a light/dark cycle of 10/14 h for one week prior to and during the experiments. The animals were fed on a standard rodent pellet diet and allowed water *ad libitum*. The rearing and upkeep of the animals throughout the experimental period was in conformity with the ethical guidelines laid down by the Institutional Animal Ethical Committee of Banaras Hindu University.

Drug treatment

The alcoholic extract was suspended in 0.5% carboxymethylcellulose in distilled water and administered once a day for three consecutive days at doses ranging from 25 mg/kg to 100 mg/kg. A 50 mg/kg oral dose of nimesulide (Dr. Reddy's, Hyderabad, India) was used as the standard anti-inflammatory drug, whereas pentazocine (Ranbaxy, Ahmedabad, India) 10 mg/kg, i.p. and aspirin (Astra-IDL Ltd., Bangalore, India) 25 mg/kg i.p., were used as the standard analgesic drugs. All the drugs were administered 30 min before the commencement of the experiment. The control group of animals received a suspension of 0.5% carboxymethylcellulose

in distilled water. The various tests were conducted on the third day, an hour after administering the last drug or vehicle.

Antinociceptive activity

Tail flick latent period: The technique described by Davies *et al.* [5] was adopted using a techno analgesiometer (Techno Electronics, Lalbagh, Lucknow, India). The cut off time for determination of latent period was taken at 30 seconds to avoid injury to the skin [6]. Three determinations of tail flick latency were done per rat at each time interval and the means of the results obtained were used for statistical analysis. Pentazocine (10 mg/kg i.p.) was used as the standard drug.

Hot plate reaction time in mice: The technique described by Woolfe *et al.* [7] was adopted. A hot plate maintained at 55 ± 1 °C was employed. The time taken for the animals to start licking their fore paws or jumping on the hot plate was taken as the reaction time. Pentazocine (10 mg/kg i.p.) was used as the standard drug.

Analgesymeter induced pain: Analgesic effect was tested in mice of either sex using an Ugo Basile analgesiometer (Ugo Basile, Varese, Italy). The method described by Rodriguez *et al.* was employed [8].

Acetic acid induced writhing response in mice: The technique described by Witkin *et al.* [9] was adopted. A significant reduction in the number of writhes in the treatment group of animals compared to the control group was considered as a positive analgesic response. The percentage inhibition of writhing was calculated.

Anti-inflammatory activity

Carrageenan-induced paw edema: The technique described by Winter *et al.* [10] was adopted using an Ugo Basile Plethysmometer (Ugo Basile, Varese, Italy). The paw volume was measured before the

injection of λ -carrageenan suspension and thereafter at 60 min intervals up to 180 min.

Statistical analysis

The results obtained were expressed as mean \pm SEM. The statistical significance of the differences between the control and treatment groups was calculated using unpaired student's t-test and Mann-Whitney U-test (two tailed). A value of $p < 0.05$ was considered significant. Results for the tail flick latency and hot plate reaction time were expressed as percentage protection (Equation 1) while those for acetic acid induced writhing test were expressed as percentage inhibition (Equation 2).

$$\% \text{ Protection} = \frac{\text{Time point of test} - \text{Zero time of test}}{\text{Zero time of test}} \times 100 \quad (1)$$

$$\% \text{ Inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100 \quad (2)$$

RESULTS

Tail flick latent period: The alcoholic extract of *P. arvensis* exhibited graded dose response and pretreatment with pentazocine significantly potentiated the antinociceptive effect of the extract (Table 1).

Hot plate reaction time in mice: Table 2 shows that the alcoholic extract significantly

increased the reaction time and provided significant protection. Pentazocine significantly increased the reaction time of *P. arvensis* extract.

Analgesymeter induced pain: The data in Table 3 indicates that mice treated with *P. arvensis* extract exhibited resistance to mechanical pain 30 min after drug administration. The weight required to elicit pain was dose dependent. Concomitant administration of the extract with aspirin resulted in synergism.

Acetic acid induced writhing: The alcoholic extract of *P. arvensis* produced a significant decrease in acetic acid induced writhing. The percentage inhibition is shown in Table 4. Under the same experimental conditions, *P. arvensis* potentiated the analgesic effect of aspirin as shown by a further decrease in the writhing response. This combination was also observed to prevent abdominal cramping.

Carrageenan induced paw edema: Treatment with *P. arvensis* produced significant and dose dependent anti-inflammatory activity at 1, 2 and 3 h. The effect was similar to that of nimesulide which in addition significantly potentiated the activity of *P. arvensis* (Table 5).

Table 1: Effect of the alcoholic extract of *P. arvensis* on tail flick latent period in rats

Treatment	Dose (mg/kg)	Mean latent period (s)		% Protection
		At 0 min	After 30 min	
Control	-	8.03 \pm 1.21	8.44 \pm 1.52	5.10
<i>P. arvensis</i>	25	8.99 \pm 1.10	10.45 \pm 1.01	16.24
<i>P. arvensis</i>	50	9.99 \pm 1.01	12.51 \pm 1.12 ^a	25.22
<i>P. arvensis</i>	100	9.94 \pm 1.32	15.45 \pm 1.32 ^b	55.43
Pentazocine	10	10.12 \pm 1.23	15.30 \pm 1.25 ^b	55.18
<i>P. arvensis</i> /pentazocine	50/10	9.58 \pm 0.90	17.83 \pm 1.61 ^b	86.11
<i>P. arvensis</i> /pentazocine	100/10	10.10 \pm 0.01	20.05 \pm 1.25 ^c	98.51

Values are mean \pm SEM, n = 6, p: ^a < 0.05, ^b < 0.01 and ^c < 0.001 compared to the control group.

Table 2: Effect of the alcoholic extract of *P. arvensis* on hot plate reaction time in mice

Treatment	Dose (mg/kg)	Mean latent period (s)		% Protection
		At 0 min	After 30 min	
Control	-	9.96 ± 1.10	10.82 ± 1.12	8.63
<i>P. arvensis</i>	50	10.15 ± 1.15	17.21 ± 2.10 ^a	69.55
<i>P. arvensis</i>	100	10.93 ± 1.32	22.64 ± 2.89 ^b	107.13
Pentazocine	10	10.45 ± 1.39	31.33 ± 4.10 ^c	199.80
<i>P. arvensis</i> /pentazocine	50/10	9.40 ± 1.05	36.54 ± 3.44 ^c	288.72
<i>P. arvensis</i> /pentazocine	100/10	9.69 ± 1.19	38.55 ± 3.83 ^c	297.83

Values are mean ± SEM, n = 6, p: ^a < 0.05, ^b < 0.01 and ^c < 0.001 compared to the control group.

Table 3: Effect of the alcoholic extract of *P. arvensis* on force-induced pain in mice

Treatment	Dose (mg/kg)	Weight causing pain (g)		% Protection
		Before administration	After administration	
<i>P. arvensis</i>	50	83.9 ± 3.72	108.1 ± 4.89 ^a	28.84
<i>P. arvensis</i>	100	84.0 ± 4.61	130.8 ± 6.05 ^b	55.71
Aspirin	10	84.2 ± 5.20	129.4 ± 6.07 ^b	53.68
<i>P. arvensis</i> /aspirin	50/25	81.3 ± 3.35	140.0 ± 6.13 ^b	72.20
<i>P. arvensis</i> /aspirin	100/25	83.1 ± 4.17	146.0 ± 7.32 ^b	75.69

Values are mean ± SEM, n = 6, p: ^a < 0.05, ^b < 0.01 and ^c < 0.001 compared to the control group.

Table 4: Effect of the alcoholic extract of *P. arvensis* on acetic acid-induced writhing in Mice

Treatment	Dose (mg/kg)	Number of writhes	% Inhibition
Control	-	24.31 ± 2.11	-
<i>P. arvensis</i>	50	17.53 ± 1.33	27.88
<i>P. arvensis</i>	100	11.15 ± 1.85 ^a	54.13
Aspirin	10	9.45 ± 1.94 ^b	61.12
<i>P. arvensis</i> /aspirin	50/25	7.87 ± 1.51 ^c	67.62
<i>P. arvensis</i> /aspirin	100/25	6.59 ± 1.21 ^c	72.89

Values are mean ± SEM, n = 6, p: ^a < 0.05, ^b < 0.01 and ^c < 0.001 compared to the control group.

Table 5: Effect of the alcoholic extract of *P. arvensis* on carrageenan-induced paw edema in rats

Treatment	Dose (mg/kg)	Paw volume (ml)			
		0 h	60 min	120 min	180 min
Control	-	1.08±0.07	1.1±0.04	1.35 ± 0.04	1.16 ± 0.03
<i>P. arvensis</i>	50	0.69±0.05	0.85 ± 0.03	0.94 ± 0.03	0.65 ± 0.02 ^c
<i>P. arvensis</i>	100	0.71±0.02	0.83 ± 0.02	0.88 ± 0.02 ^b	0.68 ± 0.01 ^c
Nimesulide	50	0.54±0.01	0.75± 0.02 ^b	0.64 ± 0.02 ^c	0.56 ± 0.01 ^c
<i>P. arvensis</i> /nimesulide	50/50	0.44±0.04	0.80 ±0.01 ^a	0.52 ± 0.01 ^c	0.47 ± 0.01 ^c
<i>P. arvensis</i> /nimesulide	100/50	0.40±0.01	0.77 ±0.01 ^b	0.47 ± 0.01 ^c	0.41 ± 0.01 ^c

Values are mean ± SEM, n = 6, p: ^a < 0.05, ^b < 0.01 and ^c < 0.001 compared to the control group.

DISCUSSION

Polygala arvensis exhibited significant antinociceptive and anti-inflammatory effects on the experimental animal models. The alcoholic extract of the plant was found to increase tail flick latency significantly. This test is useful for discriminating between centrally acting opiate and non-opiate analgesics and gives a positive response with the former only. The extract of *P. arvensis* exhibited analgesic activity in rats and potentiated the analgesic effect of pentazocine. This test has been found suitable for the evaluation of centrally, but not peripherally acting analgesics. The validity of this test has been confirmed even in the presence of substantial impairment of motor performance [11].

This study shows that *P. arvensis* may offer new perspectives in the treatment of pain. Flavonoids and tannins have been reported to produce analgesic and anti-inflammatory activities [12-14]. In the acetic acid induced writhing test, *P. arvensis* extract significantly inhibited abdominal constriction and potentiated the activity of aspirin. Acetic acid increases the levels of PGE₂ and PGF_{2α} in peritoneal fluids which may involve, in part, action at peritoneal receptors [15-16]. This is a very sensitive

method for screening the antinociceptive effect of compounds [17].

Similarly the alcoholic extract of *P. arvensis* exhibited significant anti-inflammatory activity in λ-carrageenan-induced paw edema in rats. Lambda carrageenan is a sulphated polysaccharide obtained from seaweeds (Rhodophyceae) commonly used to induce acute inflammation. Its mode of action is believed to be biphasic. The first phase is due to release of histamine and serotonin while the second phase is caused by the release of bradykinin, proteases, prostaglandins and lysosomes [18]. It has been reported that the second phase of edema is sensitive to most clinically effective anti-inflammatory drugs and has been used frequently to assess the anti-edematous effect of natural products [19-20]. Prostaglandins play a major role in the second phase of edema which sets in by the third hour of the test [21]. Based on these reports, it can be inferred that the inhibitory effect of *P. arvensis* on carrageenan-induced inflammation in rats might be due to inhibition of production of mediators responsible for inflammation and pain. The present study shows that *P. arvensis* possesses antinociceptive and anti-inflammatory activities.

ACKNOWLEDGEMENT

The authors express a deep sense of gratitude to the University Grants Commission of the Government of India for providing the financial assistance to carry out this study.

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