ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF ETHANOLIC EXTRACT OF *PSEUDARTHRIA VISCIDA* (L) ROOTS

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Summary

The present study was designed to investigate the analgesic and anti-inflammatory effects of the ethanolic extract of *pseudarthria viscida* Linn (EEPV). Acetic acid-induced writhing and hotplate tests were used to determine analgesic effect. Anti-inflammatory activity of EEPV was demonstrated with Wistar rats using carrageenan induced edema of the hind paw of rats. It has been shown that the EEPV (200/400 mg/kg, p. o.) significantly reduced acetic acid-induced writhing response, as well as significantly increase the hot-plate pain threshold in mice. In the Carrageenan induced mouse paw edema test, the group treated with indomethacin showed the strongest inhibition of edema formation by 62.50% in the third hour after carrageenan administration, while EEPV (200/400 mg/kg) showed 42.69% and 55.76% respectively. The extract did not produce any mortality up to the dose of 2000 mg / kg. Preliminary Phytochemical screening revealed the presence of flavonoids, proteins, tannins, and phenolic compounds. The result justifies the traditional uses of *Pseudarthria viscida* for the treatment of fever, inflammatory and painful conditions.

KEYWORDS- *Pseudarthria Viscida,* Analgesic, Anti-inflammatory, Carrageenan, Flavonoids.

Introduction

Pseudarthria viscida (L.) Wight and Armott (Fabaceae) is distributed throughout the South India up to 900 meter in the hills and also in Gujarat. The plant is perennial viscid pubescent semi erect, diffuse undershurb, 60-120 cm long with slender branches, more or less clothed with whitish hair. Plant is used in the preparation of Ayurvedic medicines namely, 'Dashamoolaristam' 'Mahanarayana Talia' and 'Anutailam' etc.[1,2] The roots are astringent, thermogenic, digestive, anthelmintic, anti-inflammatory, diuretic, aphrodisiac, nervine tonic. They are useful in vitiated conditions of cough, bronchitis, asthma, tuberculosis, helminthiasis, diarrhea, inflammation, cardiopathy, fever, gout, diabetes, hyperthermia and general debility.[1-6] The extracts of leaf, root, stem and callus obtained from p. viscid showed significant inhibitory activity against some fungal pathogens causing major diseases in crop plants and stored food grains.[7] It is proved also to be antioxidant and antihypertensive.[8]

Although some scientific investigations have been undertaken to validate the local uses of this plant, there seems to be no report on the analgesic and anti-inflammatory activities of the roots of the plant. Therefore this study was undertaken to investigate the analgesic, anti-inflammatory effects of the ethanol extract of the *Pseudarthria viscida* roots.

Material and Methods

Plant material

The plant *Pseudarthria viscida* was collected from Kerala and was taxonomically identified by the botanical survey of India, southern circle, TNAV Campus, Coimbatore where a voucher specimen (BSI-166) is deposited for future reference. The root was shade dried and then coarsely powdered. The coarse powder (200 g) was first defatted with petroleum ether 60–80 ◦C (1.5 L) and then successively extracted with ethanol (1.5 L) using soxhlet apparatus. The ethanol extract was concentrated at reduced temperature and pressureusing rotary evaporator to remove the solvents. The yield of ethanol extract was 5.2%. [9]

Preliminary photochemical screening

EEPV was studied for its preliminary phytochemical screening for the detection of various plant constituents.[9]

Animals

Wistar rats (150-200gm) and Swiss albino mice (20-25 gm) of either sex obtained from the laboratory animal center of the college J. K. K.Nataraja College of Pharmacy, Erode, India were used in studies. The animals were kept in standard cages with good ventilation, free access to feeds and water. Laboratory investigations on the animals were carried out in accordance with the ethical guidelines stipulated by the institutional animal ethics committee.

Chemicals and drugs

The following chemicals and drugs were used: Carrageenan (Sigma-Aldrich), acetic acid (Merck Chemicals Ltd), aspirin (Vikas Pharma, Mumbai).

Acute toxicity study (LD_{50})

The acute toxicity of EEPV was evaluated in Swiss albino mice (20-25gm). Overnight fasted mice were divided into four groups including three experimental and one control group each consists of six mice. EEPV was administered orally at the dose of 500, 1000, 2000 mg/kg to three experimental groups and control group received vehicle. The animal observed continuously for the initial 2 hrs, for its general behavior and intermittently for next 20 hrs for its mortality. There was no mortality because of extract treatment and the extracts did not show any toxicity symptoms up to 2000 mg/kg in albino mice by oral route.[10]

Analgesic activity

Acetic acid-induced writhing response in mice

The test was carried out using the described technique of Koster *et al.*[11] The EEPV (200/400 mg/kg, p.o.) or aspirin (200 mg/kg) was administered to mice, one hour before injected intraperitoneally with 0.1 ml/10 g body weight of 0.7% acetic acid solution in saline. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movements) was counted for the next 15 min, starting on the 5th min after the acetic acid injection. The data represent average of the total number of writhes observed.

Control mice received drugless (0.1% w/v CMC; 10 ml/kg) vehicle.[12] Analgesic activity was calculated as percentage maximal possible effect (MPE) using the fallowing formula.
% MPE = 100 X (mean of writhes in control group) – (mean of writhes in treated group/ mean of writhes in control group).

Tail Flick method
The mice used for this study were divided into four groups, two groups received the extracts (EEPV200 and 400 mg/kg), while the remaining two groups received saline (control) and aspirin (200 mg/kg). The extracts, saline or aspirin were administered orally to the animals after 12 h of fasting. The animals were each placed on a hot plate maintained at 55 ◦C, 30 min after administration of extracts, saline or aspirin. The time taken for the mice to respond to the thermal stimulus (usually by jumping) was noted as the latency (in second). The mean of the latency for each group was determined.[13]

Anti-inflammatory activity
Anti-inflammatory activity of Pseudarthria viscida was determined by carrageenan induced paw edema.[14] All the animals were divided into group of six animals each. First group received 1 ml of normal saline, second group received 10 mg/kg indomethacin, and group 3 and 4 received EEPV extract 200 and 400 mg/kg respectively. After 1 hour the rats were challenged with subcutaneous injection of 0.1 ml 1% w/v solution of carrageenan into the sub planter side of the left hind paw. A digital vernier caliper used for measuring paw thickness (mm) of rats. The paw volume was measured immediately after injection (0 hr) and then first, second and third hour after injection of histamine to each group. The difference between the initial and subsequent reading gave the actual edema volume. Percent inhibition of inflammation was calculated using the formula.

$$\text{Percent inhibition of inflammation} = 100 \text{ X } \frac{V_c}{} \left\{ 1 - \frac{V_t}{} \right\}$$

Where "V_t" represents oedema volume in test compound and *"*V_c*"* represent oedema volume in control.

Statistical analysis
The data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.01$ was considered as statistically significant. Data are reported as mean ± SEM.

Results

Acute toxicity studies
In Acute Toxicity study the given *Pseudarthria viscida* roots extract did not show any mortality up to the dose of 2000 mg / kg. The Extract Shows mild muscle relaxant property and diarrhea.

Preliminary phytochemical screening
Preliminary Phytochemical screening revealed the presence of flavonoids, proteins, tannins, and phenolic compounds.

Table 1: ***Effects of EEPV in acetic acid induced writhing response test***

Treatment	Dose	Number of writhing	% inhibition
Control	10 ml/kg	50.50±1.35	–
EEPV	200 mg/kg	29.17±1.16	42.2 %
EEPV	400 mg/kg	20.33±1.17	59.7 %
Aspirin	200 mg/kg	15.83±1.07	70 %

Values are mean ± S.E.M. (n = 6). $P < 0.01$ vs. the control.

Table 2: ***Effects of EEPV in hot-plate test***

Procedures	Dose	Latency period (S)
Control	-	3.31±0.22
EEPV	200 mg/kg	4.19±0.09
EEPV	400 mg/kg	4.42±0.18
Aspirin	200 mg/kg	5.15±0.11

Values are mean ± S.E.M. (n = 6). $P < 0.01$ vs. the control.

Analgesic effect

Table 1 demonstrated the pain behavior of writhing response which was presented as cumulative abdominal stretching response. The treatment of animals with EEPV (200 and 400 mg/kg, p.o.) produced a significant and dose depend inhibition in abdominal writhes produced by acetic acid. Aspirin (200 mg/kg, p.o.) exhibited inhibitory effect on writhing response. The result of the hot plate test show that oral administration of EEPV (200–400 mg/kg) produced significant increase in the latency time from 3.31±0.22 to 4.42±0.18 s after 30 min of its administration. Aspirin also increased the latency time (Table 2).

Anti-inflammatory effect

The result obtained as mean increase in paw volume (mm) and % inhibition is represented in table 1. Subplantar injection of carrageenan in rats showed to a time-dependent increase in paw thickness; this increase was observed at 1h and was maximal at 3hr after administration of carrageenan injection in the vehicle treated groups .However, carrageenan-induced inflammation was significantly ($p<0.05$) reduced in all phases of experiment by treatment with EEPV 400 mg/kg and indomethacin. The lower dose of extract (200 mg/kg) did not show any considerable change in paw odema as compared with vehicle treated group (Table 3).

Table 3: ***Anti-inflammatory activity of EEPV in carrageenan- induced paw oedema***

Groups	Mean paw volume ± S.E. (mm)				% inhibition of oedema
	1 hr	2 hr	3 hr	4hr	
Control	8.05±0.28	9.18±0.21*	10.05±0.25*	10.4±0.23*	-
Indomethacin (20 mg/kg)	7.01±0.15**	6.13±0.24*	5.83±0.24*	3.90±0.16*	62.50%
EEPV (200 mg/kg)	7.35±0.29**	6.96±0.18*	6.45±0.17*	5.96±0.17*	42.69%
EEPV (400 mg/kg)	6.88±0.18*	6.06±0.18*	5.81±.17*	4.60±0.23*	55.76%

Values are mean ± S.E.M. (n = 6).* $P < 0.01$, ** $P < 0.05$ vs. the control group.

Discussion

The results of present study indicate that the EEPV at the doses of 200–400 mg/kg produced significant anti-nociception in the acetic acid induced writhing and hot plate tests. The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesic. The writhing induced by chemical substance is due to sensitization of nociceptors by prostaglandins.[15,16] This response is thought to involve peritoneal receptors.[17, 18] The treatment of animal with EEPV (200 and 400 mg/kg, p.o.) showed a significant inhibition of the writhing which was similar to that of the effect produced by Aspirin (200 mg/kg, p.o.). In the hot-plate test, the analgesic effects were observed (Table 2), indicating that the EEPV possess analgesic activities related to non-inflammatory pain. Substances and drugs that produce strong inhibitory effects in the hot plate test can inhibit centrally induced pain and they act as strong analgesics.[19] The inhibitory effects of EEPV extracts on the two animal models suggest that they both have peripheral and central analgesic activities. Carrageenan-induced paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of

apparent systemic effects.[20] Moreover, the experimental model exhibits high degree of reproducibility. Development of edema in the paw of the rat after injection of carrageenan is a biphasic event. The initial phase observed during the first hour is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosome.[21] Present study indicates that EEPV (400 mg/kg, p.o.) and indomethacin play a crucial role as protective factors against the carrageenan-induced acute inflammation.

On preliminary phytochemical screening EEPV was found to contain Flavonoids compounds. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception.[22] Hence the presence of flavonoid may be contributory to the anti-inflammatory and analgesic activity of root of *Pseudarthria viscida.*

Based on these results, we concluded that oral administration of EEPV results in an analgesic and anti-inflammatory effect. Thus confirms the traditional use of this plant root as a therapeutic agent in inflammatory and painful condition. Finally further studies may be directed at characterizing the actual bioactive ingredients that are responsible for the observed activities in the plant.

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