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Research Article

PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL EVALUATION OF *PSEUDARTHRIA VISCIDA* LINN

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ABSTRACT

Pseudarthria viscida had been widely used for its reported biological activities in indigenous system of medicine. The present investigation was carried out to find the effect of ethanol extract of *Pseudarthria viscida* (EEPV) for its anti-inflammatory activity in rat. In this study, the anti-inflammatory activity of *Pseudarthria viscida* was evaluated by using a carrageenan-induced rat paw edema model and compared with that of standard drug Indomethacin. Oral administration of the extract at the doses 200 and 400 mg/kg b.w. exhibited dose dependent and significant anti-inflammatory activity in carrageenan-induced hind paw edema of inflammation. Both the dose of *Pseudarthria viscida* promoted the anti-inflammatory activity significantly when compared to the standard drug. Hence, present investigation established some pharmacological evidences to support the folklore claim that *Pseudarthria viscida* is used as anti-inflammatory agent.

Keywords: Anti-inflammatory activity, Carrageenan Induced paw edema model, Indomethacin**,** *Pseudarthria viscida*.

INTRODUCTION

The herbal medicines are getting more importance in the treatment of inflammation because of the toxic effect of the current therapy used to treat those inflammation using synthetic drugs. Herbal medicines are less toxic and less costly when compared to the synthetic drugs. The present study will help the industry to produce herbal drug with less side effect, less costly affordable and more effective in the treatment of inflammation. Finally the phytochemical screening or elucidation of the bioactive compounds from the plant would be effective drug against inflammation[1].

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation. Edema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow. Several experimental models of paw edema have been described. Carrageenan induced paw edema is widely used for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation whereas prostaglandins are detectable in the late phase of inflammation[2].

The plant *Pseudarthria viscida* Linn (family: Fabaceae) is useful in vitiated conditions of pitta and vata, cough, bronchitis, asthma, tuberculosis, helminthiasis, dyspepsia, inflammation, cardiopathy, haemorrhoids, gout, hyperthermia and general debility[3-5]. The plant has shown to possess antifungal[6], antioxidant[7], anti-tumor[8], anti hypertensive[9] and antidiarrhoeal[10] activities. Since no information is available on the anti- inflammatory activity of *Pseudarthria viscida,* the present study was undertaken to investigate the anti- inflammatory activity of ethanol extract of *Pseudarthria viscida* (EEPV).

MATERIALS AND METHODS

The plant *Pseudarthria viscida* Linn. (Family: Fabaceae) was collected from Kolli hills, Namakkal District, Tamilnadu, India. The plant material was taxonomically identified by the botanical survey of India, Southern circle, TNAU Campus, Coimbatore, Tamilnadu (NO/BSI/SC/5/23/06-07/tech-166).

Preparation of the extract

The whole plant of *Pseudarthria viscida* Linn was dried under shade, and made in to a coarse powder with a mechanical grinder. The coarse powder was passed through sieve no: 40 and stored in an airtight container for further use. The dried powder material was defatted with petroleum ether (60-80ºC) by using soxhlet extractor to remove waxy substances and chlorophyll, which usually interfere in the isolation of phytoconstituents. The marc, defatted with petroleum ether was dried and extracted by using ethanol (99.9 %v/v) in a soxhlet extractor for 72 hr. The solvent was then distilled off and the resulting semisolid mass was dried in a dessicator to get a yield of **4% w/w.**

Phytochemical analysis of the extract

The extract was screened for the presence of various constituents employing standard screening tests[11]. Conventional protocols for detecting the presence of secondary metabolites such as glycosides, saponins, flavonoids, tannins were used. Several phytoconstituents like flavonoids, terpenoids and tannins were present which is known to promote anti-inflammatory process due to their antioxidant activities.

Experimental animal

All the experiments were carried out according to the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi, India and approved by Institutional Animal Ethical Committee. (Regd. No: 997 /c /06 / CPCSEA).

Wistar rats of either sex weighing 150-200g were used for the study. On arrival, the animals were placed randomly and allocated to treatment groups in poly propylene cages (47×34×18cm) with paddy husk as bedding. It was renewed every 24h. Animals were housed at a temperature of 24 ± 2°C and relative humidity of 30-70% and light: dark (12:12h) cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted chew (M/s. Hindustan Lever Ltd., Mumbai). The standard pellet diet comprised 21% protein, 5% lipids, 4% crude fibre, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamin and 55% nitrogen free extract (carbohydrates). It provides metabolisable energy of 3,600 kcal.

Acute Oral Toxicity study of EEPV

The EEPV treated animals were observed continuously for the initial 2 hrs for its general behavior, intermittently up to 24 hrs for its mortality (short term toxicity) and up to 14 days for long term toxicity[12-14]. The animals did not show any mortality up to the dose level of 2000 mg/kg body weight in any of the groups and were considered as safe. Hence 2000mg/kg body weight was considered as MTD (Maximum Tolerated Dose), 1/10th and 1/5th of the value of

MTD were taken as treatment dose for further studies (200mg/kg and 400mg/kg).

Anti-inflammatory effect of EEPV by Carrageenan induced paw edema method

The rats were divided into four groups. The extract of the EEPV and standard used for this study were prepared. Animals were deprived of food and water for 18 h before the commencement of an experiment. On the day of the experiment each group is assigned with 6 rats. They were marked and numbered for identification. The first group received 0.5% carboxy methyl cellulose (CMC) (10 ml/kg, orally), while the second group received Indomethacin (10mg/kg, orally). The third and fourth group were treated with EEPV (200 mg/kg and 400 mg/kg, orally). The doses of extracts were selected on the basis of acute toxicity test. A mark was made on both the hind paws just below the tibio-tarsal junction so that every time the paw could be dipped in the mercury column of plethysmometer up to the mark to ensure constant paw volume. Thirty minutes after treatment, an inflammatory edema was induced in the left hind paw by injection of 0.1 ml of carrageenan (1% w/v) in the plane tissue of the paw of all the animals. The right paw served as a reference to inflammed paw for comparison. The relative increase in the paw volume was measured by using plethysmometer in control, standard and sample treated groups in the time duration of 1, 2, 3, 4 and 5 hrs after carrageenan injection. The degree of edema formation was assayed by the percentage increase in paw volume i.e., edema rate (E) in animals treated with standard drug and the treated with EEPV. These were compared with the increased paw volume of control animals[15-18].

The ratio of the anti-inflammatory effect of EEPV was calculated by the following equation

$$\text{Anti- Inflammatory activity (\%)} = (1\text{-}D/C) \times 100$$

Where, D represents the percentage difference in paw volume after EEPV was administered to the mice and C represents the percentage difference of volume in the control group.

Statistical Analysis

All the results were expressed as mean ± standard error mean (S.E.M). Data were analyzed statistically by using one-way ANOVA followed by Dunnet's test. The minimum level of significance was set at $P < 0.05$. All the analysis was conducted in triplicate and statistical analysis by using Graph pad prism software of version 5.

RESULTS

In the present study anti-inflammatory activity of ethanol extract of *Pseudarthria viscida* on Carrageenan induced paw edema method was studied. The paw volume of Carrageenan induced rats at different time interval is given in Table 1 and Graph 1. And Percentage inhibition on paw edema at different time interval is given in the Table 2 and Graph 2.

Table 1: Evaluation of Anti inflammatory effect of EEPV on Carrageenan induced paw edema method

Groups	Paw Volume (ml)				
	1 hr	2 hr	3 hr	4 hr	5 hr
Control CMC	0.55 ± 0.07	1.18 ± 0.11	1.41 ± 0.09	1.43 ± 0.07	1.39 ± 0.07
Indomethacin	0.43 ± 0.09	0.46 ± 0.08***	0.58 ± 0.12*	0.62 ± 0.09***	0.62 ± 0.08***
EEPV (200mg/kg)	0.45 ± 0.05	0.81 ± 0.06***	0.96 ± 0.08***	0.99 ± 0.09**	0.94 ± 0.12**
EEPV (400mg/kg)	0.41 ± 0.06	0.63 ± 0.07***	0.78 ± 0.15**	0.79 ± 0.12**	0.63 ± 0.09***

Results are expressed in Mean ± SEM; * P<0.05, **P<0.01 and ***P< 0.001.

Graph 1:

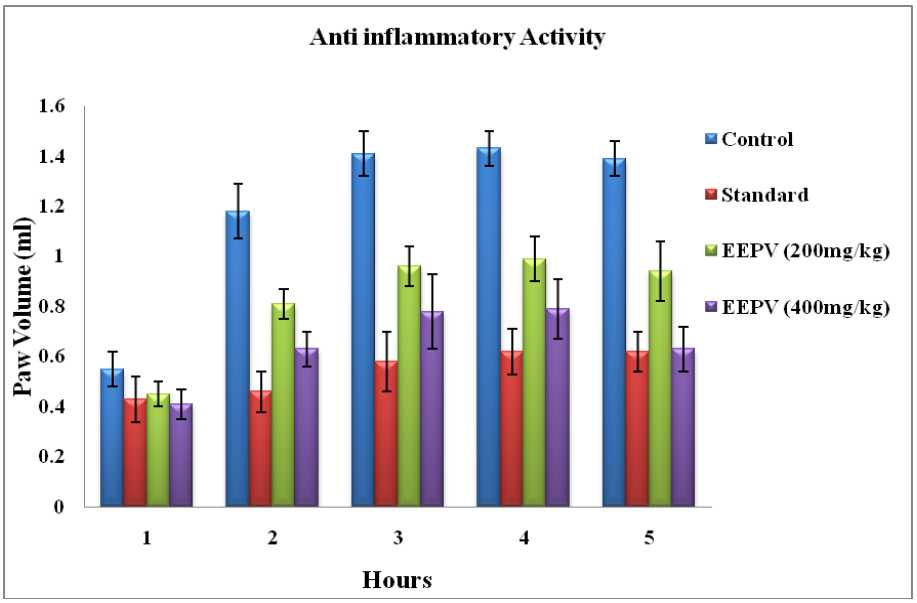
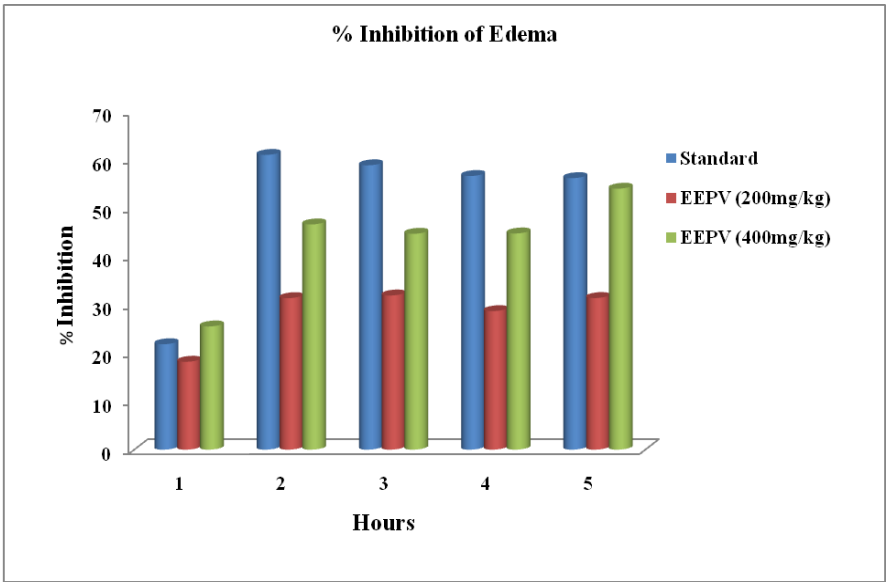


Table 2: Comparative study of Percentage Inhibition of EEPV on Paw edema

Groups	% Inhibition of Paw Edema				
	1 hr	2 hr	3 hr	4 hr	5 hr
Indomethacin (10 mg/kg)	21.82	61.01	58.86	56.64	56.20
EEPV (200mg/kg)	18.19	31.36	31.91	28.67	31.38
EEPV (400mg/kg)	25.5	46.62	44.68	44.75	54.01

Graph 2:



The result shows the effect of EEPV on Carrageenan induced paw edema in rats. Significant decrease in paw edema was observed in EEPV 200mg/kg (18.19-31.91%) and 400 mg/kg (25.5-54.01%) when compared with the control group. The anti-inflammatory activity was dose dependent and was found to be statistically significant at the higher concentration 400 mg/kg when compared with the activity of indomethacin (21.82-58.86%), a standard drug Indomethacin.

DISCUSSION

The Carrageenan induced paw edema test is widely accepted as a sensitive acute model for investigation of the potential anti-inflammatory agents particularly for non-steroidal anti inflammatory drugs. In this test, development of inflammation is a biphasic event with a maintenance phase. EEPV showed inhibition of inflammation in both phases. The initial phase (1-2 hr) is primarily mediated mainly by histamine and serotonin. The EEPV had moderate anti-histaminic activity by inhibiting histaminergic receptors and there by it may inhibit the initial phase of edema test. As earlier reported that EEPV contained flavanoids and terpenoids, which are known to impair histamine release from the mast cells and exert anti-inflammatory effects.

Further, the edema maintained between the first and second phase (2-3 hr) is due to kinin like substances specially bradykinin. The second phase is linked to the release of prostaglandins, arachidonic acid metabolites, neutrophils migration, proteolytic enzymes as well ass other neutrophils derived mediators[19-20]. The EEPV also contain flavanoids and tannins which impair cycloxygenase or lipooxygenase enzyme activities that would reduce the levels of prostaglandins and other arachdonic acid metabolites[21]. Such a mechanism may account for impairment of the late phase inflammation by EEPV.

CONCLUSION

It is concluded that ethanol extract of *Pseudarthria viscida* possess significant anti-inflammatory activity against experimentally induced paw oedema in rats. This may be due to the presence of reported active Phytoconstituents & their influence on the prostaglandins pathway. Further research, to isolate anti-inflammatory principle & exact mechanism involved, is needed.

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