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Evaluation of anti-inflammatory effects of *Pergularia Daemia* latex using experimental animal models

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Abstract
Pergularia Daemia (Apocynaceae) plays an important role in traditional ayurvedic and siddha for the treatment of asthma, bronchitis, and jaundice. Interestingly, the latex of (PDL) is applied to cure *Pergularia Daemia* headache, migraine, boils, wounds and scabies. The present study is aimed to evaluate the anti-inflammatory efficacy of PDL in animal. Orally, pre-treated three different doses (100, 200 mg/kg) of PDL in order to evaluate distinct phases of anti-inflammatory effects.
The inhibition ratio of the PDL (200 mg/kg, ($P < 0.01$)) on carrageenan, Phorbol 12-myristate 13-acetate (PMA) in rats were 66.46 and 54.04% respectively better than the inhibition ratio of indomethacin (5 mg/kg) were 52.31% and 70.33% respectively in the two model. PDL sustains exemplary anti-inflammatory activity.

Keywords: *Pergularia Daemia,* apocynaceae, anti-inflammatory, antinociceptive, opioid system

Introduction
Inflammation is an intricate pathophysiological process mediated by array of signalling molecules produced by leukocytes, macrophages and mast cells response of living tissue to undesirable stimuli. The displacement of leukocytes from blood to tissue leads plasma protein extravasation at inflammatory site and Macrophages release NO, Prostaglandins and pro-inflammatory mediators (TNF-α, IL-6, IL-1B) [1]. Mast cells secrete numerous vasoactive peptides and pro-inflammatory mediators such as histamine, serotonin, TNF, kinins [2]. These pro inflammatory mediators cause severe pain has become a complicated field. The known compounds from natural pharmacophores and synthetic compounds still struggle with side effects. Unfortunately, side effects such as gastric lesions caused by NSAIDs, the use of these drugs as anti-inflammatory agents have not been successful in all the cases. As a result, promising new anti-inflammatory drugs are seeking as a replacement to NSAIDs [3].
Pergularia Daemia (forsk.) is a perennial herb which belonging to family Apocynaceae, are growing extensively along shrub areas of tropical and subtropical regions in India [4]. *Pergularia Daemia* has *Pergularia Daemia* long been engaged in traditional ayurvedic and siddha medicine in folk remedies for treating head, ear, eye and tooth ache [5]. Precisely the latex is practiced to cure Migraine, boils, Lymph glands, ringworm, scabies and leucoderma [6, 7]. Leaf juice and latex are used in diarrhoea, asthma and applied in rheumatic swellings [8]. Till now, most researches on focus multiple pharmacological *Pergularia Daemia* properties and characterise the phytochemicals derived from various parts of the plant unless latex [4]. In this study *Pergularia Daemia* was primarily directed to evaluate the different models of anti-inflammatory activities *in vivo*.

Materials and Methods
Experimental animals
Male and female Swiss albino mice (25-30 g) and Wistar rats (250-280 g) were used. Animals were maintained under standard

conditions (i.e. at 26 ± 2 ºC, humidity: 45-50% and 12 h natural light/dark cycle) and fed with standard pellet diet and distilled water ad libitum. Each of these treatment groups were classified as six animals/ group. The protocol of the study was approved by the Institutional Animal Ethics Committee (IAEC) of Sree Datta College of Pharmacy, constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), approval no. CPCSEA/2022/1636/PO/Re/S/0021.

Plant identification and latex collection

Latex of *Pergularia Daemia* was collected from around the areas of Ranga Reddy District, Telangana, India. The plant identification and authentication was done by Dr. Madhav Chetty Assistant Professor, Sri *Venkateswara University*, *Tirupati*, Andhra Pradesh. A voucher specimen (BSI/SRC/5/23/2022/TECH/1674) was deposited at the Herbarium of the Department.The collected latex (500g) was dried in oven at 35 ºC to obtain 30% dry powder.

Preparation of Extract (PDL& PDLFR)

About 125 g of *Pergularia Daemia* latex was exhaustively extracted on Soxhlet using Petroleum ether as a solvent. The defatted semidried latex powder 100 g was classified into two parts. The small (30 g) portion was extracted with 90% aqueous methanol for 3 h. The extracts were filtered and concentrated by using rota vapour and lyophilized to yield the crude (11 g) (PDL).

Acute Oral Toxicity Study of PDL

As stated by OECD guidelines-425 [9], Wistar rats were classified into four groups of six each. Group I, standard control was treated with distilled water whereas; Group II is the toxic group, the groups III and IV were orally treated with PDL in different doses of 200 and 2000 mg/kg, respectively. After the 14 days of observation period, the animals were euthanized and histological studies were conducted on kidney tissues of the animals using haematoxylin-eosin (H&E) staining. Photomicrographs were taken with light microscope (Lab vision I-3000) at 40X.

Carrageenan-induced Paw Oedema (an acute inflammation model)

The male rats were partitioned into five groups of six each.

Group I: Control group administered with 10 mL/kg distilled water. Group II: Positive control administered with Indomethacin (5 mg/kg) via p.o. 1 h before the carrageenan injection [10]. Groups III and IV were dosed p.o. with 100 and 200 mg/kg of PDL dissolved in water, respectively, after 1 h of the administration of doses.

The 0.1 mL of 1% carrageenan in saline was injected into the subplantar region in the left hind paw of the rats. Paw volumes were determined by using Plethysmometer (Ugo Basil, Italy) at times of 1, 3 and 4 h post carrageenan administration.

Phorbol 12-myristate 13-acetate (PMA) induced mouse ear edema activity

According to a modified method of [11] 4 µg per ear of PMA, in 20 µl of acetone, was applied to both surfaces of the right ear of each mouse. The left ear (control) received the vehicle (acetone and/or DMSO, 20 µl). The selected plant extract was administered topically (50 and 100 mg per ear in DMSO) 1 h before PMA application. Two control groups were used: a control group with the application of PMA on the right ear and the reference group was treated with indomethacin (2 mg per ear in 20 µl acetone). Six hours after PMA application, mice were killed by cervical dislocation and a 6 mm diameter disc from each ear was removed with a metal punch and weighed. Ear edema was calculated by subtracting the weight of the left ear (vehicle) from the right ear (treatment), and was expressed as edema weight. Inhibition percentage was expressed as a reduction in weight with respect to the control group.

Statistical Analysis

Results were expressed as mean ± SEM. The statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's and Tukey's multiple comparison tests. Values less than 0.001 ($p < 0.001$) and 0.05 ($P < 0.05$) were considered as indicative of significance. The IC50 values were calculated from the dose/response nonlinear regression plots by using Graph pad prism 5.0.

Results

Acute toxicity study

In the acute oral toxicity test with high dose 2000 mg/kg of PDL does not produce mortality in mice during the observation for 14 days. The mice are not exhibiting any signs of toxicity, aberrant behaviour or other physiological activities.

Carrageenan-induced Paw Oedema (an acute inflammation model)

The anti-inflammatory and anti-nociceptive potential of PDL was tested by using chemical and physical models in which, carrageenan induced acute inflammation is believed to be bi-phasic. The initial phase (1-3 h) is mediated by the early release of histamine and serotonin followed by the release of kinins and extensively releases bradykinin, prostaglandins (PGs) and lysosome. The later phase is proclaimed to be sensitive to both steroidal and non-steroidal anti-inflammatory agents clinically [12].

The anti-inflammatory activity of the PDL against acute pedal oedema has been shown in Table 2 which showed significant anti-inflammatory activity and the results were comparable to that of control. It was observed that the PDL (200 mg/kg, p.o.) exhibits maximum anti-inflammatory activity against Carrageenan induced hind paw edema. The inhibition obtained with PDL was 66.46 % (Table 1).

The PDL influences the both phases of carrageenan induced

inflammation caused by the suppression of Prostaglandins synthesis inhibition, antihistamine and serotonin activities which stimulate nociceptors and thus induce pain. The inhibitory effect of PDL on carrageenan induced inflammation may be due to inflammatory mediators.

Table 1: Anti-inflammatory activity of PDL against carrageenam induced Paw Oedema

Group	Mean increase in paw volume (mL)				% Decrease in paw volume at 3 h
	0 hr	1hr	2hr	3 hr	
Group I	0.94 ± 0.01	1.53 ± 0.007	1.89 ± 0.004	2.49 ± 0.007	
Group II	0.90 ± 0.008	1.05 ± 0.01*	1.30 ± 0.002**	1.61 ± 0.001*	52.31
Group III	0.96 ± 0.037	1.25± 0.035**	1.51 ± 0.32**	1.71 ± 0.049**	50.31
Group IV	0.95 ± 0.046	1.19± 0.061**	1.32 ± 0.037**	1.46 ± 0.035**	66.46

N= 6, treatment, mg/kg, data were analyzed using ANOVA and expressed as Mean ± SEM followed by Dunnett's test and differences between means were regarded significant at * [P<0.05], ** P<0.01.

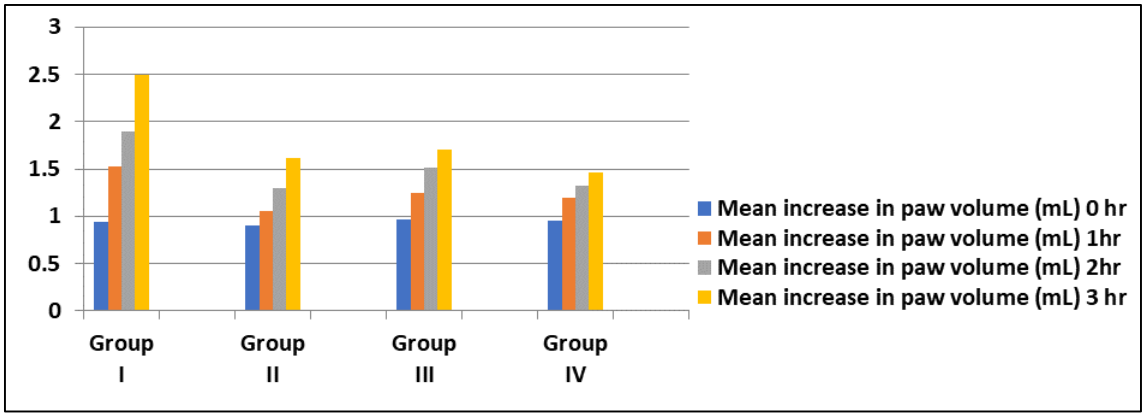

Fig 1

4.5. Oedama induced by PMA in mouse ear activity

It can be seen in Table 2 that the selected plant samples inhibit PMS induced inflammation in mouse ear. The extracts of PDL (54.06 %) shows significant ($P < 0.05$) inhibitory results as compare to PMA treated group. Indomethacin was used as reference compound also possess an excellent inhibitory activity.

Table 2: Effect of plant samples on edema induced by PMS in mouse ear

Group	Treatment	Dose (mg/ear)	PMA- induced ear edema	
			edema weight (mg)	% Inhibition
Group I	PMA	0.004	16.45 ± 2.41	
Group II	Indomethacin	5 mg/kg via p.o	04.88 ± 1.98	70.33
Group III	PDL	100	10.89 ± 0.78*	33.79
Group IV	PDL	200	07.56 ± 1.89**	54.04

* (P < 0.05), ** (P < 0.01) statistical significance compared with PMA groups (One Way ANOVA for multiple comparison test followed by dunnet test).

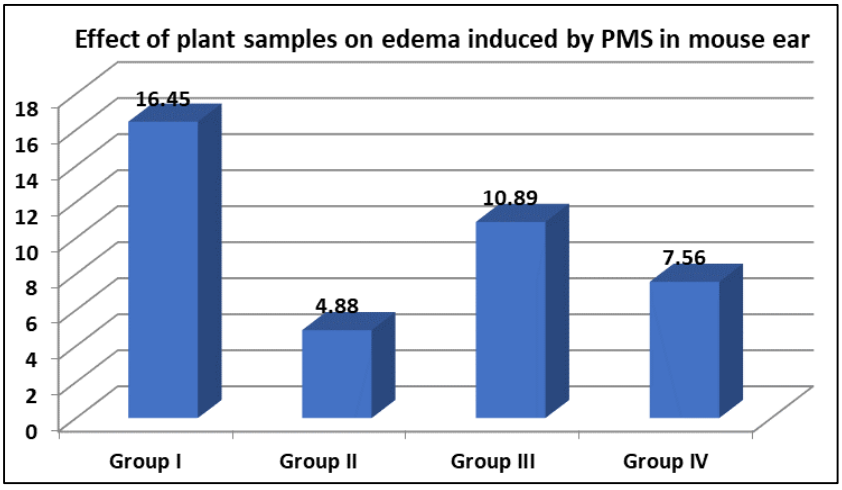

Fig 2

Discussion

Developing novel, effective and safe anti-inflammatory agents has remained a major thrust area in the main stream of 'finding alternatives to NSAID's. Anti-inflammatory agents possessing selective COX-2 inhibition and showing no or negligible effect on COX-1 activity are more appreciated as safe drugs as these agents have minimum gastrointestinal side effects. Natural product, especially medicinal plants and drug discovery has remained a very successful combination for the inventorization of new therapeutic agents.

Variety of phytochemicals like flavonoids, terpenoids, alkaloids and saponins has been described to possess significant anti-inflammatory activity. Several studies proved that naturally occurring coumarins [13] and flavonoids [14] act as dual inhibitors of cyclooxygenase and 5- lipoxygenase activities. The Indian spice turmeric, (*Curcuma longa* L.) possessing curcumin (and synthetic analogs) have established reputation as an anti-inflammatory agent by inhibiting COX-1 and COX-2 [15]. Flavonoids inhibit biosynthesis of prostaglandins (the end products of the COX and lipoxygenase pathways), which acts as a secondary messengers and are involved in various immunologic responses [16]. Inhibition of these enzymes provides the mechanism by which flavonoids inhibit inflammatory disorders [17].

Fewyears back, highly effective class of novel anti-inflammatory drugs such as Celecoxib, Rofecoxib, and Valdecoxib etc. were introduced in the pharmaceutical market but unfortunately most of them were withdrawn from the market on account of their serious cardio functioning side effects, especially in high sensitive patients like pregnant women, new born children, elderly people etc. [18, 19]

Worldwide, there is an increasing concern in finding new anti-inflammatory remedies not only having improved therapeutic index but also harmless. The results of the COX inhibition studies focus the importance of selected botanicals as an important resource for the isolation and identification of new COX-2 selective anti-inflammatory agents.

Pergularia Daemia is a medicinal herb, well known for its pain-relieving efficacy in traditional ayurvedic and siddha medical systems. Specifically, the latex is exercised against migraine, head and earache. So far, no studies have been reported to entertain the role of anti-inflammatory effects of the latex. In this research work, *in vivo* anti-inflammatory studies were primarily conducted in animal models to evaluate the anti-inflammatory potential of *P.daemia* latex. The inhibition ratio of the *P.daemia* latex extract (200 mg/kg) on carrageenan and PMA-induced ear oedema in rats are 66.46% and 54.04% respectively. Therefore, the role of the latex from *P.daemia* as anti-inflammatory drugs in animal models recommends it as a traditional analgesic.

Conclusions

In summary, these findings assist the role of PDL in traditional medicine as pain and inflammation drugs. Interestingly, the outcome of the present study adverts that PDL possesses remarkable central and peripheral antinociceptive effects in animal models that are possibly mediated both by inhibition of pro-inflammatory mediators generation and activation of an opioidergic mechanism. Moreover, the antinociceptive activity of PDL might be imputed to the presence of fatty acid amides and polyphenolics (flavonoids) based compounds.

Therefore, more precise studies are required to identify the bio active compounds which have the potential to exert an opioid anti nociceptive activity at the central and peripheral level could be a good competitor for the evolution of new analgesic drug that is lack of dependence, tolerance and addiction effects as morphine.

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