

## BIOLOGICAL EVALUATION OF IN-VIVO ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF *PAEDERIA FOETIDA* ROOT EXTRACT

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### ABSTRACT

The ethanolic root extract of *Paederia foetida* was evaluated for its analgesic and anti-inflammatory activities using different models of Pain and Inflammation. The analgesic activity of *Paederia foetida* was carried out using tail-flick, eddy's hot plate and acetic acid induced writhing response in mice. Further in-vivo anti-inflammatory study was performed using carrageenan- induced rat paw edema in rats. The ethanolic root extract of *Paederia foetida* at a dose level of 100 and 200 mg/kg were tested for its therapeutic potential. The results were found to be encouraging in all animal models and the results were tabulated in detailed. *Paederia foetida* root extract possess significant analgesic and anti-inflammatory activities against various models of inflammation and pain.

**KEYWORDS:** *Paederia foetida*, Analgesic and Anti-inflammatory, Tramadol, Diclofenac Sodium, Carrageenan.

### INTRODUCTION

Inflammation is a pathophysiological response of a living tissue that leads to local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism that helps body to protect itself against infection, burns, toxic chemicals or other noxious stimuli, complex events and mediators involved in the inflammatory reaction, all these may induce or aggravate many diseases.<sup>[1]</sup>

*Paederia foetida* belongs to the family Rubiaceae<sup>[2]</sup> and the root of the plant is not well explored for its biological activities and the plant has been used in folk medicine for the treatment of inflammation,<sup>[3]</sup> aches and piles.<sup>[4]</sup> The natives of Maredumalli, Rampachodavaram and Jaddangi forest areas are using this plant as a traditional medicine for wide variety of ailments. In our research study the root of the plant was subjected to various experimental protocols.

### MATERIALS AND METHODS

#### 1. Collection and Authentication of the Plant

The roots of *Paederia foetida* were collected from Maredumalli, Rampachodavaram and Jaddangi villages of East Godavari districts of Andhra Pradesh in the month of August and September 2021. The plant was identified and authenticated by taxonomist Prof. Dr. T.

U. Raghuram of Maharani Government Degree College, Peddapuram. The roots were chopped into small pieces, coarsely powdered and soaked in ethyl alcohol for 72 hrs. Later it was subjected to extraction process and the extract obtained was filtered and evaporated to dryness in vacuum at ordinary temperature, the extract so obtained is used for the evaluation of biological studies.

#### 2. Preparation of the Extract

The pulverized coarse powder of the roots of *Paederia foetida* (250gm) was extracted with ethyl alcohol, the extract so obtained was evaporated to dryness at low temperature and kept in a desiccator for 3 days.

#### 3. Experimental Animals

Albino rat's 150-200grams and albino mice 30-50grams of either sex were used in the entire study. They were housed in standard polypropylene cages and kept under room temperature at 30 °C relative humidity of 70% in light/dark cycle, the animals were fed with standard diet and water ad libitum. Food was withdrawn 12 hour before and during the experimental hours. The experimental protocol was approved by institutional animal ethical committee with Reg no: 1269|P<sub>0</sub>|E|S|08|CPCSEA.

#### 4. Acute toxicity studies

The acute toxicity of test extract was determined in albino mice of either sex weighing between 30-50grams. The animals were divided into different groups comprising 3 animals each. The control group received the vehicle and the other groups received 100, 200, 400, 800, 1600mg/kg of extract respectively. After administration with different doses of extract the animals were carefully observed for 72 hours (acute study) for their gross behavioral changes and for 14 days (chronic study). No toxicity or death was observed in the experimental rats. There were no significant toxic signs or death during the entire observation period. Then LD<sub>50</sub> was calculated by OECD 425.<sup>[5]</sup>

#### 5. Screening of analgesic activity

**a) Tail-flick method:** Albino mice weighing 30-50grams were divided into four groups each with 3 mice. Animals were kept in cages for a period of time for getting adapted to laboratory environment surroundings and were fed with food & water.<sup>[6]</sup>

**Group-1** Animals administered with 0.2ml/N saline.

**Group-2** Animals administered with root extract of *Paederia foetida* (100mg/kg)

**Group -3** Animals administered with root extract of *Paederia foetida* (200mg/kg)

**Group-4** Animals administered with standard drug Morphine sulphate (5mg/kg- S.C)

#### b) Eddy's Hot Plate Method

Albino mice weighing between 30-50 grams were divided into 4 groups of 3 mice each. Animals were housed in cages & were given a period of time to get adapted to laboratory environment surroundings and were fed with food and water.

**Group-1** Animals administered with 0.2ml/N saline.

**Group-2** Animals administered with root extract of *Paederia foetida* (100mg/kg)

**Group -3** Animals administered with root extract of *Paederia foetida* (200mg/kg)

**Group-4** Animals administered with standard drug Tramadol (5mg/kg- I.P)

#### c) Acetic-acid induced writhing response in mice

The writhing syndrome was elicited by Intraperitoneal injection of acetic acid(0.1 ml of 1% Solution) and No. of writhes displayed from 5-20mins were recorded<sup>[7]</sup> The extracts of *Paederia foetida* 100 & 200 mg/kg were administered orally in their respective doses 1 hour prior to the test.

$$\text{Percent inhibition of writhing} = (1 - wt/wc) \times 100$$

Where Wc and Wt represents the average number of writhing produced by the control and test group, respectively.

#### d) In-vivo Carrageenan induced paw edema

The anti-inflammatory activity of *Paederia foetida* root extract was assessed using carrageenan induced rat paw edema protocol. Carrageenan inflammation was induced by injecting 0.1 ml of 1% carrageenan into the sub-plantar tissue of the right hind paw<sup>8</sup>. The root extract of *Paederia foetida* was suspended in gum acacia and administered at doses of 100 & 200 mg/kg oral respectively, 1 hour prior to the carrageenan injection. The paw volume was measured at the intervals of 0,1,2,3 and 4 hours using plethysmometer.<sup>[9]</sup>

#### Statistical Analysis

Results have been indicated in terms of means  $\pm$  SEM. Differences between the groups were statistically determined by ANOVA with Dunnett's multiple comparison test using Graph pad instat version of 5.00, Graph pad software.

## RESULTS AND DISCUSSION

### Tail flick method

**Table 1: Effect of *Paederia foetida* root extract in mice using Tail Flick method.**

Treatment Group	0 mins		15 mins		30 mins		60 mins		120 mins	
	B.R.T	% Inhibition	B.R.T	% Inhibition	B.R.T	% Inhibition	B.R.T	% Inhibition	B.R.T	% Inhibition
Group-I Control	5.6 $\pm 0.1$	-	5.9 $\pm 0.3$	-	5.5 $\pm 0.9$	-	5.2 $\pm 0.02$	-	5.4 $\pm 0.8$	-
Group-II <i>Paederia foetida</i> [100mg/kg]	3.5 $\pm 0.4$	-	4.9 $\pm 0.1$	36.5	7.8 $\pm 0.2$	42.1	7.4 $\pm 0.4$	40.2	6.5 $\pm 0.9$	32.2
Group-III <i>Paederia foetida</i> [200mg/kg]	5.5 $\pm 0.9$	-	7.7 $\pm 0.1^*$	48.2	8.5 $\pm 0.9^*$	68.0	7.6 $\pm 0.3^*$	54.3	7.0 $\pm 0.3^*$	45.6
Group-IV Std Drug- Morphine Sulphate [5mg/kg]	6.3 $\pm 0.8^*$	-	9.3 $\pm 0.3^{**}$	54.5	9.5 $\pm 0.7^{**}$	78.0	8.0 $\pm 0.2^{**}$	64.0	7.5 $\pm 0.5^*$	52.3

Data analyzed using one way analysis of variance (ANOVA) and expressed as Mean ± SEM (n=3) followed by Dunnett’s test and differences between the means were considered as significant at P ≤ 0.05

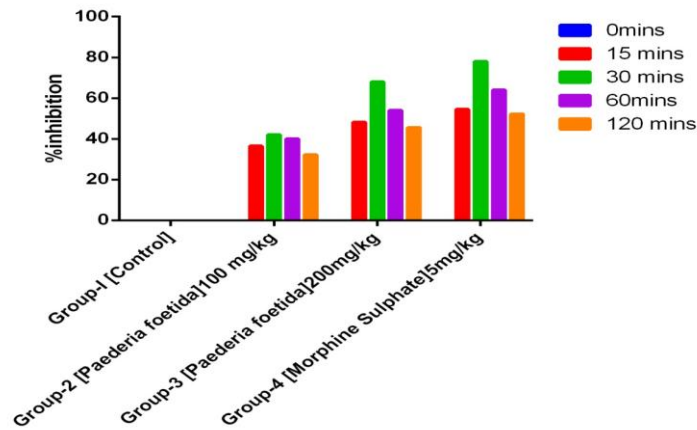


Fig. 1: Effect of *Paederia foetida* root extract %inhibition of pain in mice.

Table-2: Effect of *Paederia foetida* root extract in mice using Eddy’s Hot Plate method.

Treatment Group	0 mins		15 mins		30 mins		60 mins		120 mins	
	B.R.T	% Inhibition	B.R.T	% Inhibition	B.R.T	% Inhibition	B.R.T	% Inhibition	B.R.T	% Inhibition
Group-I Control	6.5 ±0.1	-	7.3 ±0.02	-	8.5 ±0.13	-	8.7 ±0.2	-	7.2 ±0.2	-
Group-II <i>Paederia foetida</i> [100mg/kg]	6.0 ±0.02	-	8.0 ±0.2	32.3	11.0 ±0.2	64.2	11.2 ±0.3	74.3	10.0 ±0.2	56.2
Group-III <i>Paederia foetida</i> [200mg/kg]	6.30 ±0.1	-	9.2 ±0.15*	40.3	11.6 ±0.17*	89.3	13.20 ±0.3*	94.5	10.40 ±0.21*	70.2
Group-IV Std Drug- Tramadol [5mg/kg]	7.5 ±0.05*	-	10.8±0.80*	48.5	13.20 ±0.3**	92.5	15.20 ±0.2**	98.4	10.60 ±0.01*	80.2

Data analysed using oneway analysis of variance (ANOVA) and expressed as Mean ± SEM (n=3) followed by Dunnett’s test and differences between the means were considered as significant at \*P ≤ 0.05.

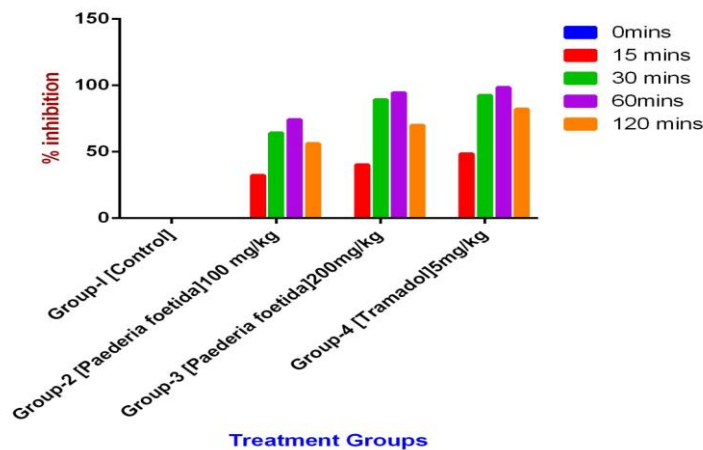


Fig. 2: Effect of *Paederia foetida* root extract in Nociceptive Inhibition using Eddy’s hot plate method.

Table-3: Effect of *Paederia foetida* root extract in mice using Acetic acid induced writhing response.

Treatment Group	No. of writhing's	% Inhibition
Group-I Control	83 ±1.46	-
Group-II <i>Paederia foetida</i> [100mg/kg]	50 ±2.3***	39.7%
Group-III <i>Paederia foetida</i> [200mg/kg]	33 ±2.3***	60.2%
Group-IV Std Drug- Morphine Sulphate [5 mg/kg]	5 ±0.32***	92.8%

Data analyzed using one way analysis of variance (ANOVA) and expressed as Mean ± SEM (n=3) followed by Dunnett's test and differences between the means were considered as significant at P ≤ 0.001.

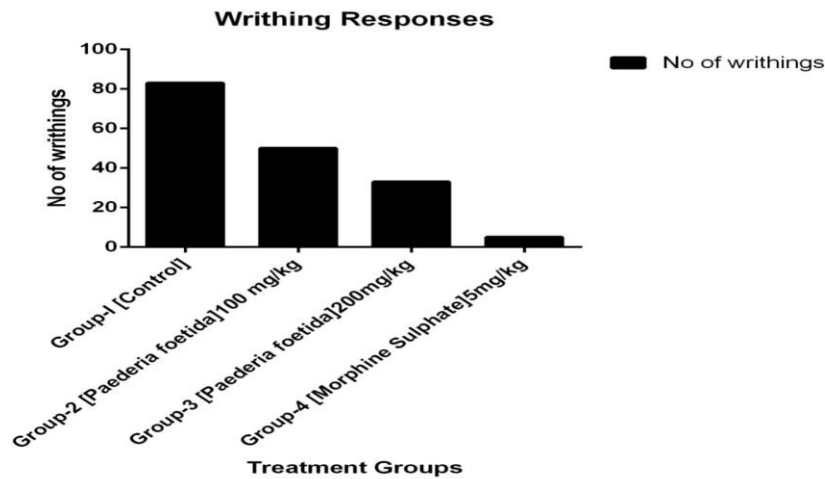


Fig-3: Effect of *Paederia foetida* extract in acetic acid induced in writhing in mice.

Table 4: Effect of *Paederia foetida* root extract on carrageenan induced rat paw edema.

Treatment Group	Mean increase in Paw volume				
	0 Hour	1 Hour	2 Hours	3 Hours	4 Hours
Group-I Control	0.30 ±0.03	0.50 ±0.02	0.90 ±0.04	1.10 ±0.15	0.74 ±0.02
Group-II <i>Paederia foetida</i> [100mg/kg]	0.28 ±0.01	0.50 ±0.01	0.74 ±0.02	0.96 ±0.01	0.60 ±0.02
Group-III <i>Paederia foetida</i> [200mg/kg]	0.32 ±0.02	0.35 ±0.02*	0.60 ±0.03*	0.80 ±0.02*	0.50 ±0.02*
Group-IV Std Drug- Diclofenac sodium [10mg/kg]	0.27 ±0.01	0.32 ±0.01	0.46 ±0.02**	0.62 ±0.01**	0.30 ±0.02**

Data analyzed using one way analysis of variance (ANOVA) and expressed as Mean ± SEM (n=3) followed by Dunnett's test and differences between the means were considered as significant at P ≤ 0.05.

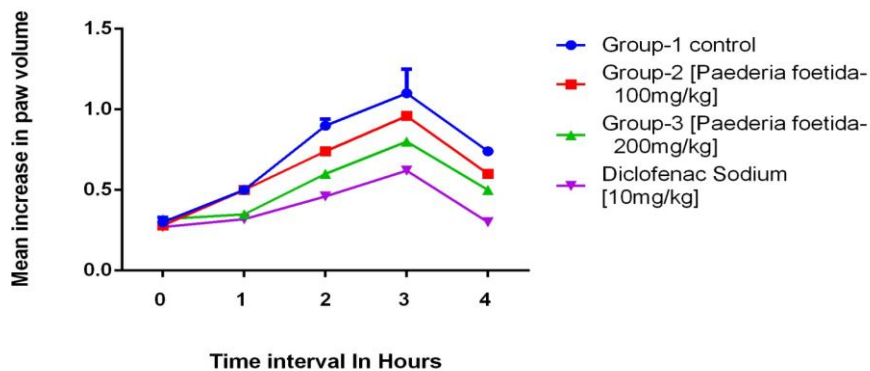


Fig. 4: Effect of *Paderia foetida* root extract showing % inhibition of carrageenan induced rat paw edema.

DISCUSSION

In the present research study, the analgesic and anti-inflammatory effects of *Paederia foetida* root extract was tested in different experimental models of pain and

inflammation to investigate whether the ethanolic root extract of *Paederia foetida* has true analgesic potential with tail flick and Eddy's hot plate methods were used. In the tail flick method *Paederia foetida* root extract at a dose of 200mg/kg produced 68% of inhibition at 30 min

as compared to Standard drug Morphine sulphate (5mg/kg) which produced 78% of inhibition at 30min.

In the Eddy's hot plate method *Paederia foetida* root extract at a dose of 200mg/kg produced 94.5% inhibition at 60 min interval when compared to Standard drug Tramadol (5mg/kg) which produced 96.8% inhibition at 60 min interval.

The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesic and the response is taught to be mediated by peritoneal mast cells<sup>[10]</sup> acid sensing ion channels<sup>[11]</sup> and the prostaglandin pathways.<sup>[12]</sup> In this method *Paederia foetida* extract at a dose of 200 mg/kg exhibited 33 writhing with 60.2% inhibition as compared to Standard drug Morphine sulphate [5mg/kg] which exhibited 5 writhing with 92.8% inhibition.

In all these methods *Paederia foetida* root extract exalted the tolerance capacity of the animals which may be the indication of possible involvement of higher center. The carrageenan induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of Non -Steroidal Anti-Inflammatory Agents, which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis<sup>[13]</sup> *Paederiafoetida* root extract at a dose of 200mg/kg reduced the paw edema induced by carrageenan and reduced the inflammation. The experimental results obtained from our research study clearly demonstrates that the ethanolic root extract of *Paederia foetida* possessed significant analgesic and anti-inflammatory properties and these might be related to the Phytochemical constituents present in the plant.

## CONCLUSION

*Paederia foetida* root extract exhibited excellent analgesic and anti-inflammatory activities due to the presence of various phytochemical constituents. However the possible isolation of these active constituents need to be studied extensively, so that possible mechanism of action and SAR could be established.

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