**Bulletin of Environment, Pharmacology and Life Sciences**
Bull. Env. Pharmacol. Life Sci., Vol 5 [5] April 2016: 68-71

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804


**ORIGINAL ARTICLE** **OPEN ACCESS**

# Analgesic and Anti-inflammatory activity of stem of *Pergularia daemia* Forsk Chiov.

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**ABSTRACT**
*The present study was carried out to find out analgesic anti-inflammatory activity of Pergularia daemia Forsk Chiov stem extract and we have also find out cytotoxicity of each extract. Pergularia daemia (Forsk.) Chiov (Asclepiadaceous) is a foetid smelling laticiferous twine, found in the plains throughout the hot parts of India. Pergularia daemia have multiple applications in different folk medicine, including the Indian Ayurvedic system. Pergularia daemia (Forsk.) stem was extracted with petroleum ether and methanol as a solvent. Analgesic and anti-inflammatory activity of these extracts were performed in rats with hot plate, tail immersion and Carrageenan induce rat paw edema and toxicity was assessed The doses used are (100mg/kg and 300mg/ kg) petroleum ether and methanol extract showed significant and dose depend analgesic and anti-inflammatory effect. These extracts do not have any acute toxicity. The study focused on the analgesic and anti-inflammatory effect of Pergularia daemia Forsk. Three major compounds present in the plant may explain these activities: triterpene (lupeol, lupeol acetate, alpha-amyrin), Phytosterols (beta-sitosterol, stigma sterol) flavonoids (quercetin).*
*Key words- Perguleria daemia, Hot plate, tail immersion, carrageenan*

Received 02.02.2016 Revised 29.02.2016 Accepted 12.03.2016

## INTRODUCTION

The plant is pungent, cooling; anthelmintic, laxative, antipyretic; cures biliousness, ulcers, useful in eye troubles, urinary discharges, leucoderma, strangury, uterine complaints, inflammations;[1] plant extract given for uterine and menstrual troubles and facilitate partituran[2]. Cough, a asthma, amenorrhea, dysmenorrheal, intermittent fever and leucoderma [3].Traditionally the plant *Pergularia daemia* is used as anthelmintic, laxative, antipyretic, infertile diarrhea, intermittent fever [4].The latex is used for toothache applied on wounds for quick relief. It is used for remedy for cold and fever5.As yet there is no any work published related to analgesic and anti-inflammatory activity of *Pergularia daemia* Forsk stem. The present study was carried out to on rats to investigate analgesic, anti-inflammatory activity of stem extract of *Pergularia daemia* Forsk.

## MATERIAL AND METHOD

### Plant material

The *Pergularia daemia* Forsk was collected from the surrounding area of railway station at Yeola, Dist Nashik in the month of September and were authenticated from Prof. Sandenshive H.O.D. Dept of Botany SSGM Arts, Commerce and Science College, Kopargaon. A voucher specimen has been deposited in dept of Botany of SSGM Arts, Commerce and Science College, Kopargaon. Also authenticate from Botanical Survey of India, Pune having voucher specimen No. (Letter no- BSI/WRC/IDE/NCER/2016/648A, Dated-22/01/2016)

### Extraction

The powder of *Pergularia daemia* [Forsk] stem was extracted by using continuous hot extraction method. The powder of *Pergularia daemia* [Forsk] stem was charged in to thimble of Soxhlet apparatus and extracted by using petroleum ether as a solvent by maintaining a temp [40-60$^0$C] extraction was continue till a color less solvent appears from siphon tube. Then the extract was concentrated and the percentage

yield was calculated. The marc was air dried and subjected to further extraction by continuous hot extraction process using methanol as a solvent by maintaining a temp [60-80$^0$C] again extraction was continue till a color less solvent appears from siphon tube. The extract was then concentrated and percentage yield was calculated. These extracts were stored in a refrigerator below 10$^0$C by naming petroleum ether extract [PEPD] and methanol extract [MPD].

**Animals**

Albino mice of either sex weighing between 25-30 gm were procured from National Toxicology Centre, Pune for experimental purpose. The animals were acclimatized to laboratory condition for 7 days. Animals have free access of water and standard pellet animal diet (Chakan oil Mill, Pune; India) *ad libitum*. All animal studies were performed in accordance to guideline of CPCSEA and Institutional Animal Ethics Committee [IAEC] of Sanjivani College of Pharmaceutical Education and Research Kopargaon, Maharashtra [CPCSEA registration no- 1093/PO/a/2007/CPCSEA]

**Drugs**

*Pentazocine* [30 mg/kg], and all chemicals of analytical grade. *Pentazocine,* and Methanol extract were dissolved in distilled water just before administration. For petroleum extract was suspended in CMC [0.5%]. A gastric catheter was used for oral drug administration. The extract did not show any sign and symptoms of toxicity till oral dose 2000mg/kg hence the extract was used in range of 100-300mg/kg orally assuming that $LD_{50}$ dose is 2000mg/kg.

**Preliminary Phytochemical screening of extract**

The extracts were subjected to preliminary phytochemical test for detection of phytoconstituents. 0.5gm of extract was dissolved in 5 ml of water then filter it and on filtrate test was performed [6].

**Determination of $LD_{50}$ of stem extract of *Pergularia daemia* [Forsk]**

Acute oral toxicity of *Pergularia daemia* [Forsk] stem extract was determined by using Swiss albino mice of either sex weigh between 25 ± 02 gm maintained under standard condition. The animals were fasted for 2-3 hr. prior to the experiment. Animals were administered with single dose of either petroleum ether or methanol extract of *Pergularia daemia* [Forsk] stem and observed for its mortality up to 48 hrs. Study period (short term toxicity) was based on short term toxicity profile; the next dose was decided as per OECD guideline No. 425. Since no mortality was observed up to dose 2000mg/kg. From the $LD_{50}$ dose, 100mg/kg and 300mg/kg dose were selected and considered as low and high doses respectively [7].

2.7 Analgesic activity by Hot plate model

The analgesic effect was studied using digital hot plate (Columbus- USA) instrument wherein the reaction time (paw licking, jumping or any other sign of discomfort) was recorded 60 minutes after administration of respective drugs as mentioned below on 1$^{st}$ (acute model) 11$^{th}$ and 21$^{st}$ day (chronic Model) The temperature of the plate was maintained at 55$^0$C 01$^0$C. A cut off reaction time of 30 second was chosen in order to avoid injury. *Pentazocin* (30 mg/kg/s.c.) was used as a reference standard and it was given only on 1$^{st}$, 11$^{th}$ and 21$^{st}$ day.

**Analgesic activity by Tail immersion method**

The analgesic effect was studied using Tail immersion model (Digital Water Bath- V J India) wherein the reaction time i.e. time taken for flicking of tail out of water was recorded 60 minutes after administration of respective drugs as mentioned below on 1$^{st}$ (acute model) 11$^{th}$ and 21$^{st}$ day (chronic Model) The temperature of the water was maintained at 55$^0$C ± 01$^0$C. A cut off reaction time of 30 second was chosen in order to avoid injury. *Pentazocin* (30 mg/kg/s.c.) was used as a reference standard and it was given only on 1$^{st}$, 11$^{th}$ and 21$^{st}$ day [8].

**Anti-inflammatory activity by Carrageenan induce rat paw odema**

36 rats were divided into 6 groups, each containing 6 rats. These rats were subjected to respective treatments as per the groups for the period of 10 days. On the 10$^{th}$ day, 1 hour after the dosing, all animals were injected with 0.1 ml of 1% Carrageenan into sub plantar region of hind paw and paw volume (mean displacement Volume) was measured at 0$^{th}$ 3$^{rd}$, 6$^{th}$ and 24$^{th}$ hour using digital plethysmometer.

**Statistical analysis**

Values are expressed as mean ± S.E.M. Statistical significance for analgesic activity and anti-inflammatory activity was calculated using one way analysis of variance ANOVA. Significant differences between mean were determine by Dunnetts (P< 0.05*, P< 0.01**, P< 0.001***)

## RESULT AND DISCUSSION

1. Preliminary phytochemical testing of stem extract shows presence of various phytoconstituents in petroleum ether extract, it shows presence of triterpene, sterols where as methanol extract shows presence of alkaloids, glycosides, flavonoids, tannins and phenolic components

2. Acute toxicity by oral administration of petroleum ether and methanol extract of *Pergularia daemia* stem did not produce any toxic effect in mice. The petroleum ether and methanol extract of *Pergularia*

*daemia* stem was found to be safe and no mortality was observed up to 2000 mg/kg. The data was analyzed with help of AOT425 Software.

3. In analgesic activity by hot plate method the results showed that PEPD 100 as well as 300mg/kg and MPD 300mg/kg posses significant analgesic activity on 11th and 21st day whereas all were not significant on day 1.

The PEPD 100 mg/ Kg showed duration dependent significant increase action whereas PEPD 300 mg/kg and MPD 300 mg/kg were found to be equipotent on 11th and 21st day. Overall PEPD 300 mg/kg was most significant dose.

4. Analgesic activity by tail immersion method

It showed that PEPDS 300mg/kg most potential dose which posses duration dependent significant analgesic activity. PEPDS 100mg/kg and MPDS 300 mg/kg showed equipotent action at 21st day. Reference standard *Pentazocin* has shown consistent action at all three days of its treatment.

5. Anti-inflammatory activity by Carrageenan induce rat paw odema

This indicate that the significant activity with higher doses at all times with both the extracts indicating some common phytochemical which are present in both these extract may be responsible for it. Results also reported that peak activity was at 6 hr indication duration dependent effect of the extract.

6. Phytoconstituent responsible for the activity

Five major phytoconstituents were present in stem of plant responsible for analgesic and anti-inflammatory activity triterpenes (lupeol, oleanolic acid, ursolic acid) flavonoid (Kaempferol, luteolin), phytosterol (stigma sterol, beta sitosterol). Most of these compounds are known for their analgesic activity as lupeol for anti-inflammatory[9] stigma sterol, beta-sitosterol, luteoline for antioxidant, atherosclerosis.[10] *Kaempferol* for anti-inflammatory and antioxidant [11].

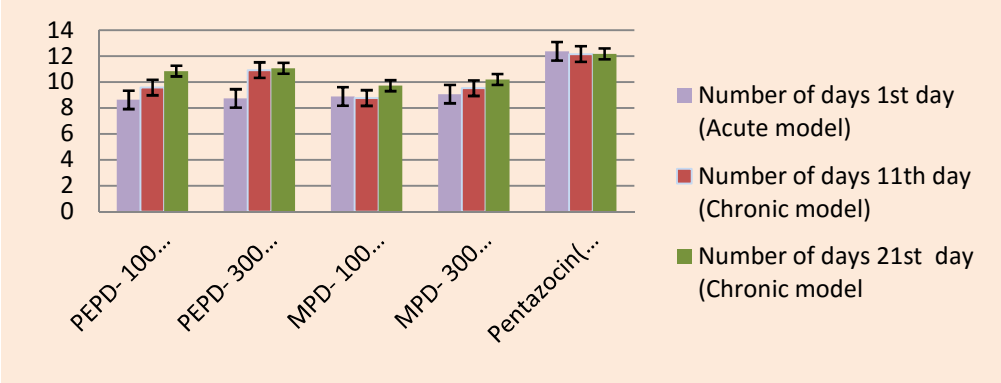


Fig. 1 Analgesic activity of *Pergularia daemia* stem in rat using the hot plate method result are expressed as ± S.D.; *P<0.05, **P<0.01,***P<0.001 compared with control(positive control Pentazocine at 30 mg/kg PEPD: Petroleum ether extract of *Pergularia daemia* Forsk; MPD methanol extract of *Pergularia daemia* Forsk

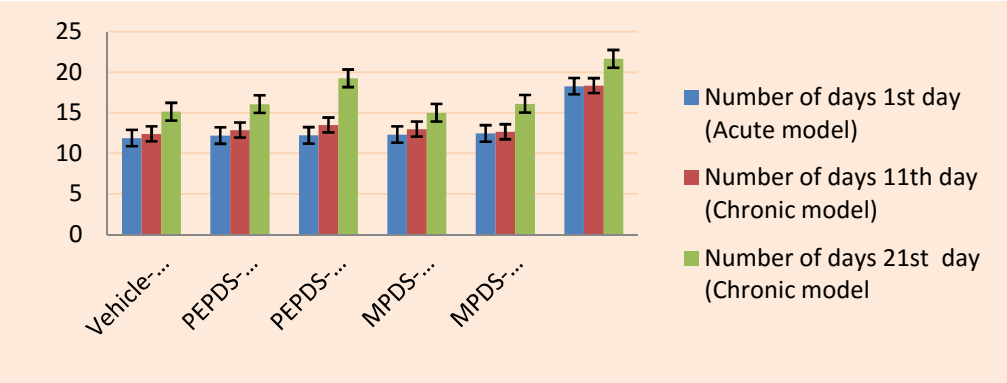


Fig. 2 Analgesic activity of *Pergularia daemia* stem in rat using the tail immersion method result are expressed as ± S.D.; *P<0.05, **P<0.01,***P<0.001 compared with control (positive control) *Pentazocine* at 30 mg/kg PEPD: Petroleum ether extract of *Pergularia daemia* Forsk; MPD methanol extract of *Pergularia daemia* Forsk

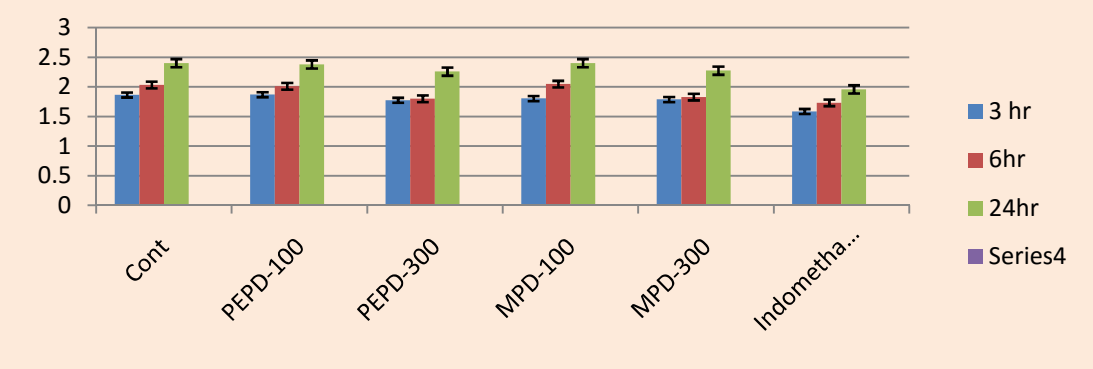


Fig. 3 Anti-inflammatory activity of *Pergularia daemia* stem in rat using the hot plate method result are expressed as ± S.D.; *P<0.05, **P<0.01,***P<0.001 compared with control(positive control *Pentazocine* at 30 mg/kg PEPD: Petroleum ether extract of *Pergularia daemia* Forsk; MPD methanol extract of *Pergularia daemia* Forsk

**ACKNOWLEDGEMENT**

Author wish to thank to Prof. Sandanshiv, HOD dept. of Botany of SSGM College Kopargaon, Prof. Veyhare N. S. for helping pharmacological activity and Prof. Arote Sir for kind support.

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**CITATION OF THIS ARTICLE**

N. G. SUTAR, S.C. PAL, Ritu Gilhotra. Analgesic and Anti-inflammatory activity of stem of *Pergularia daemia* Forsk Chiov. Bull. Env. Pharmacol. Life Sci., Vol 5[5] April 2016: 68-71