

Original Article

EVALUTION OF ANTIARTHRITIC ACTIVITY OF LEAF EXTRACTS OF PERGULARIA DAEMIA [FORSK] PLANT IN EXPERIMENTAL ANIMALS

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ABSTRACT

**Objective:** *Pergularia daemia* [Forsk] has been used from the long time in traditional medicine. The main objective of this work is to evaluate anti-rheumatic activity of leaf of *Pergularia daemia* [Forsk]

**Methods:** Anti-arthritic activity of petroleum ether and methanol extracts of leaf of *Pergularia daemia* [Forsk] was evaluated by using Freund's complete adjuvant model.

**Results:** Preliminary Phytochemical investigation of Petroleum ether extract of leaf of *Pergularia daemia* [Forsk] shows presence of steroids, terpenoids, sterols while methanolic extract of leaf of *Pergularia daemia* [Forsk] shows presence of alkaloids, glycosides, flavonoids, tannins and phenolic component. The petroleum ether and methanol extract of leaf of *Pergularia daemia* [300 mg/kg] shows an improvement in arthritis condition by reducing hind paw inflammation.

**Conclusion:** The methanolic extract was found to be more potent than petroleum ether extract. The result indicates that the petroleum ether and methanol extract contain such phytochemical constituents which are responsible for analgesic and anti-rheumatic activity.

**Keywords:** *Pergularia daemia* [Forsk] Freund's complete adjuvant model, *Methotrixate*.

INTRODUCTION

Rheumatoid arthritis, one of the commonest autoimmune disease, it is chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joints destruction, which further may be responsible for deformity and disability especially in substantial socioeconomic impact and hence need to addressed at all times [1,2] over all it involves complicated pathogenesis with pathological changes in multiple targets [3] Complete Freund's adjuvant [CFA] induce arthritis an experimental model which consider closest to stimulating human rheumatoid arthritis. The appearance of secondary lesions (uninjected paw swelling) is the manifestation of cell mediated immunity (T cell responses particularly CD<sub>4</sub><sup>+</sup> T cells). Pain is an unpleasant sensory emotional experience associated with actual or potential tissue damage, or described in terms of such damage [4].

Although these drugs are widely used for reliving pains but are associated with numerous untoward effects like hyperacidity, gastric lesions caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as anti inflammatory and analgesic agents have not been ideal in all the cases. Therefore, alternate analgesic and anti-inflammatory drugs without serious side effects are being searched all over the world. During this process, the investigation of the efficacy of plant based drugs used in traditional medicine has been paid great attention. Folk medicine and ecological awareness suggest that they usually cost less than synthetic drugs and undesirable side effects are less frequent [5]

Plant introduction

The plant *Pergularia daemia* [Forsk] (Asclepiadaceae) [6] known as "Veliparuthi" in Tamil, "Uttaravaruni" in Sanskrit and "Utranjutuka" in Hindi. Traditionally the plant *Pergularia daemia* is used as anti-helminthic, laxative, antipyretic and expectorant, also used to treat infertile diarrhea and malarial intermittent fevers [7-9]. Latex of this plant used for toothache [10]. Stem bark remedy for cold [11]. and fever [12]. Aerial parts of this plant the various pharmacological activities like hepatoprotective [13]. antifertility [14]. Anti diabetic

[15] analgesic, anti-pyretic and anti-inflammatory. Phytochemically the plant has been investigated for cardenoloids, alkaloids, and saponins [16]. The plant was found to contain various triterpenes and steroidal compounds [17]

MATERIAL AND METHODS

Plant Material

The fresh leaves of *Pergularia daemia* [Forsk] was collected from the area of railway station near to Yeola (August 2013), which was identified and authenticate by Taxonomist Prof. S. E. Saindanship, H. O. D. Department of Botany of SSGM college of Arts, Commerce and Science, Kopargaon. The fresh stem was collected dried and cut in smaller pieces as per requirement, remainder was powdered.

Extraction

The leaf powder of *Pergularia daemia* [Forsk] was extracted by using continuous hot extraction method. The leaf powder of *Pergularia daemia* [Forsk] was charged in to thimble of Soxlet apparatus and extracted by using petroleum ether as a solvent by maintaining a temp [30-40°C] extraction was continued till a colour 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°C] again extraction was continued till a colour less solvent appears from siphon tube. Then the extracted was then concentrated and percentage yield was calculated. These extracts were stored in a refrigerator below 10°C by naming petroleum ether extract [PEPD] and methanol extract [MPD].

The petroleum ether and methanol extract of *Pergularia daemia* [Forsk] were subjected to following study

1. Preliminary Phytochemical study.
2. Pharmacological activity.
  - a. Acute toxicity study.
  - b. Anti-arthritic activity.

## Animals

Albino mice of either sex weighing between 25-30 gm and albino rats of either sex weighing between 180-220 gm were procured from National Toxicology Centre Pune for experimental purpose. The animals were acclimatized to laboratory condition for 7 days. Animals 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 Health Committee [IAEC] of Sanjivani College of Pharmaceutical Education and Research Kopergaon, Maharashtra [CPCSEA registration no-1093/PO/a/2007/CPCSEA,

## Drugs

*Methotrexate* [0.75mg/kg] all chemicals of analytical grades, *Methotrixate*, and Methanol extract were dissolved in distilled water just before administration. 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<sub>50</sub> dose is 2000mg/kg.

## Preliminary Phytochemical screening of extract

The extracts were subjected to preliminary phytochemical test for detection of phytoconstituent. 0.5 gm of extract was dissolved in 5 ml of water filter it and the filtrate test were performed [18].

## Determination of LD<sub>50</sub> of leaf extract of *Pergularia daemia* [Forsk]

The acute oral toxicity of leaf extract of *Pergularia daemia* [Forsk] was determined by using Swiss albino mice of either sex weigh between 25 ± 02 gm maintained under standard condition. The animals were fasted for 3 hr. prior to the experiment. Animals were administered with the single dose of either petroleum ether or methanol leaf extract of *Pergularia daemia* [Forsk] and observed for

its mortality up to 48 hrs. study period (short term toxicity). 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<sub>50</sub> dose, 100mg/kg and 300mg/kg dose were selected and considered as low and high doses respectively [19].

## Assessment of anti-arthritic activity

### Anti-arthritic activity

Thirty six preselected male rats (having mean displacement value without any significant difference) were divided in six groups each consisting of 06 rats. All rats were made arthritic by single intradermal injection of 0.1 ml of Complete Freund's adjuvant (CFA) containing 1.0 mg dry heat-killed *Mycobacterium tuberculosis* per milliliter sterile paraffin oil into a foot pad of the left hind paw of rats and immediately after this following treatment was started for the period of next 21 days. [20].

## Results

Preliminary Phytochemical analysis of Petroleum ether extract of leaf of *Pergularia daemia* [Forsk] shows the presence of Steroids, sterols, terpenoids where as methanol extract of leaf of *Pergularia daemia* [Forsk] shows presence of alkaloids, glycosides, tannins, flavonoids and phenolic component.

### Acute toxicity study

Both petroleum ether and methanol extracts did not produce any sign and symptoms of toxicity.

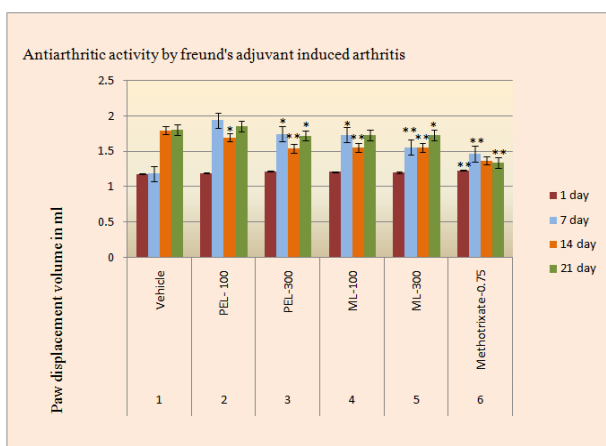
### Assessment of anti arthritic activity

60 minutes after dosing, the left hind paw volumes of displacement using digital plethysmometer and appearance as well as severity of secondary lesions were measured on 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day. Thereafter Radiographic analysis investigations were carried as a supportive parameters using the procedure mentioned below.

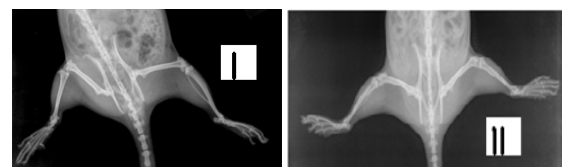
**Table 1: Secondary lesions observed due to effect of Petroleum ether and Methanol extract of leaf of *Pergularia daemia* [Forsk] as anti-arthritic activity by Freund's adjuvant induced arthritis.**

Gr. No. Treatment	Secondary lesions	1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
1	Vehicle- 10 ml/kg	---	+	++	+++
2	PEPD-100 mg/kg	---	+	++	+++
3	PEPD-300 mg/kg	---	+	+	++
4	MPD- 100 mg/kg	---	+	++	+++
5	MPD- 300 mg/kg	---	---	+	+
6	Methotrexate 0.75 mg/kg	---	---	---	+

Nil: ---; Mild: +; Moderate: ++; Severe: +++, **Note:** Secondary lesions were collectively observed in the ear, fore-paws, hind-paws and tail of rats (Newbould, 1963).



**Fig. 1: Effect of Petroleum ether and Methanol extract of leaf of *Pergularia daemia* [Forsk] as anti-arthritic.**



Control Std- Methotrexate



Sample- Methanol extract

**Photographs. 1, 2, 3 are of Anti-arthritic activity of Effect of Petroleum ether and Methanol extract of leaf of *Pergularia daemia* [Forsk]**

### Radiographic Analysis

On the 21<sup>st</sup> day immediately after measurement of paw volume displacement and recording of secondary lesions, the one rat from each group was sacrificed by cervical dislocation and carotid bleeding and subjected to the radiological examination using Agfa digital System and Siemens X ray machine. These radiographs were evaluated for any deformity especially for soft tissue swelling and bone erosion, joint space narrowing by independent qualified person. [21]

Rheumatoid arthritis is one of the commonest autoimmune diseases prominently manifested by the joint pain and inflammation has reported a large number of mortality and morbidity and there by left substantial socioeconomic impact [2] the currently synthetic drugs containing steroids are mainly used for symptomatic relief and side effect associated with it. As there are so many difficulties and hence there is a demand for alternative medicine. With these difficulties the field of arthritis research area has become a prominent thrust area. Modern research in the field of anti-arthritis treatment directed towards developing potent components with wide acceptance, no side effects and ability to suppress immune response to an antigen. The anti rheumatic activity of leaf of *Pergularia daemia* [Forsk] using Complete Freund's adjuvant induced arthritis model was performed. The research work reported that a higher dose of MPD shows that improvement in arthritis condition by reducing hind paw inflammation. MPD is comparatively more potent than petroleum ether extract when compared with standard. There is reduction in secondary lesions on MPD treated animals on 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>th</sup> day. This result suggests that the MPD extract give symptomatic relief and also used as the preventive remedy, which have more importance as compare to modern therapy.

Radiographic analysis is considered to be the best tool to screen the any drug in this regard. In present study radiographic analysis of the joint showed significant prevention in progress of joint [20, 21]. By synthetic therapy, there is rapid reduction in inflammation in arthritis is observed with corticosteroids but the effect of these drugs were for short time. Corticosteroids become less effective over time where as arthritis is usually active for years together [22]. From the above research work, the leaf extract of *Pergularia daemia* [Forsk] is a good substitute for corticosteroids therapy for arthritis.

### DISCUSSION

The preliminary phytochemical screening of PEPD shows the presence of Steroids, sterols, and MPD show the presence of alkaloids, glycosides, flavonoids, triterpenes, and tannins. Thus, the anti-rheumatic activity of *Pergularia daemia* [Forsk] may be due to sterols, alkaloid and flavonoids components. The result of the present study indicate petroleum ether and methanol leaf extract of *pergularia daemia* [Forsk] possess antirheumatic activity which in accordance with ethanomedical use. antirheumatic effect of extract was demonstrated in an experimental model using the complete Freund's adjuvant induced arthritis model.

Complete Freund's adjuvant induced arthritis has been used as a model of sub-chronic or chronic inflammation in rats and is of considerable relevance after the study of pathophysiological and pharmacological control of inflammatory process, as well as the evaluation of analgesic potential or anti-inflammatory effects of drugs (Butler et al., 1992; Besson and Guilbaud, 1988). One of the reasons for the wide utilization of this model is due to the strong correlation between the efficiency of therapeutic agent in this model and in rheumatoid arthritis in humans. Secondly, Freund's adjuvants are commonly used because they produce a stronger, longer lasting immunogenic response compared to other adjuvants. They are easy to use water-in-oil emulsions. Freund's Complete Adjuvant is the form that contains killed cells of *Mycobacterium butyricum* to enhance the immune response. CFA administration produced arthritis in two phases an acute particular inflammation followed by a phase of bone involvement (Jacobson et al., 1999). Freund's adjuvant into the rat paw induces inflammation as primary lesion

with a maximum after 3 to 5 days. Secondary lesions occur after a delay of approximately 11 to 12 days which are characterized by inflammation of non-injected sites (hind leg, forepaws, ears, nose and tail), a decrease of weight and immune responses. Anti-inflammatory compounds do not inhibit secondary lesions, which are prevented or diminished by immunosuppressive agents

### CONCLUSION

The methanolic extract of leaf of *Pergularia daemia* [Forsk] showed beneficial activity as a long term therapy in rheumatoid arthritis.

### CONFLICT OF INTERESTS

Declared None

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