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Research Article

ANALGESIC ACTIVITY OF AQUEOUS AND ALCOHOL ROOT EXTRACTS OF *PERGULARIA DAEMIA* (FORSK.) CHIOV.

LOKESH T. NIKAJOO

Olive Lifesciences Pvt Ltd, #32, 4th Floor, Anjana Complex, 2nd Main Road, Vyalikaval, Bangalore-560003, India
Phone- +91 - 080 – 64521494, Fax- +91 - 080 - 41699382
E-mail: lokesn@gmail.com; saklokesh@gmail.com

ABSTRACT

Pergularia daemia (Forsk.) Chiov. (Asclepiadaceae) is used in *Ayurveda* for the treatment of inflammation, fever, strangury, asthma, diseases of *vata* and *kapha*. It is known as Uttaravaruni and Yugaphala in *Sanskrit.* The present study was undertaken to evaluate the analgesic activity of the aqueous and alcohol root extracts of *Pergularia daemia* (Forsk.) Chiov. using eddy's hot plate and heat conduction method. In eddy's hot plate method the aqueous extract showed significant analgesic activity at the doses of 500 mg/kg ($p<0.01$) and 1000 mg/kg ($p<0.001$) and alcohol extract showed significant analgesic activity at the doses of 500 and 1000 mg/kg ($p<0.001$). In heat conduction method both extracts showed significant analgesic activity at the doses of 500 & 1000 mg/kg ($p<0.001$) as compared to control group, when analyzed statistically by Tukey Kramer Multiple Comparison Test.

The result obtained show that the aqueous and alcohol root extracts of *Pergularia daemia* (Forsk.) Chiov. possesses significant analgesic activity which confirms the traditional claims of the plant mentioned in *Ayurveda*.

Keywords: *Pergularia daemia*, Asclepiadaceae, Root, Analgesic activity.

INTRODUCTION

Pergularia daemia (Forsk.) Chiov. (Asclepiadaceae) is a foetid smelling laticiferous twiner found in the plains throughout the hot parts of India, ascending to an altitude of 1000 m in the Himalayas[1]. *Pergularia* species are widely distributed in the old world tropics and subtropics from southern and tropical Africa and Asia, have multiple applications in different folk medicine, including the Indian *Ayurvedic* system, and have been documented for antifertility[2], wound healing[3], antidiabetic[4], hepatoprotective[5], cardiovascular effect[6], antibacterial activity[7]. The plant is useful in the diseases of vatha, convulsion, asthma, poisoning; the root is useful in mental disorder, anaemia, leprosy and piles[8]. Plant possesses stomachic, laxative and diuretic properties, useful in cough, biliousness and sore eyes. Leaf paste mixed with castor oil is applied to joints in inflammation, liver complaints, spleen enlargement; leaves have hypoglycemic activity[9]. The juice of the leaves is given in asthma and applied to rheumatic swellings in combination with lime or ginger; it is also used in the preparation of medicinal oil given in rheumatism, amenorrhoea, and dysmenorrhoea[10].

The plant has been investigated phytochemically for cardenolides, alkaloids, triterpenes and saponins[11]. Ethanol extract of aerial parts of *Pergularia daemia* (Forsk.) Chiov. reported for anti-inflammatory, antipyretic, analgesic activity[12]. Earlier reports on biological activities of root extract are scarce. Therefore, the present study was undertaken with the objective to investigate the analgesic activity of the aqueous and alcohol root extracts of *Pergularia daemia* (Forsk.) Chiov. in a scientific manner using Swiss albino mice.

MATERIALS AND METHODS

Plant material

The roots of *Pergularia daemia* (Forsk.) Chiov. were collected from the forest of Savanadurga, Bangalore, India. The identification and authentication was carried out at Department of Pharmacognosy, M.S. Ramaiah College of Pharmacy, Bangalore by Dr. S.N. Yoganarasimhan (Taxonomist and Research Co-ordinator).

A voucher herbarium specimen (*Lokesh Nikajoo* 006) in flowering and fruiting condition along with a voucher sample of the crude drug material was deposited at the herbarium and museum.

Preparation of extracts

Fresh roots of *Pergularia daemia* (Forsk.) Chiov were washed, shade dried, powdered, passed through a #60 mesh sieve and were extracted with alcohol (95% v/v) in a soxhlet apparatus by continuous heat extraction. The extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50ºC. The alcohol extract was prepared in distilled water containing 2% v/v Tween 80 (as a suspending agent) for experimental purpose.

The aqueous extract was prepared by maceration in chloroform water. The macerate was filtered through Whatman No.1 filter paper and concentrated in a rotary flash evaporator at a temperature not exceeding 50ºC.

Phytochemical analysis

Phytochemical analysis of different extracts was carried out by successive solvent extraction. Weighed quantity of air dried powdered roots was extracted in soxhlet apparatus successively with solvents started with petroleum ether (60°-80°C) followed by benzene, chloroform, acetone and alcohol (95% v/v). After extracting with each solvent, the marc was dried in hot air oven below 50ºC; finally the marc was macerated with chloroform water for 24 hour. Each extract was concentrated by distilling off the solvent and evaporating to dryness. The dry extracts were subjected to preliminary phytochemical screening for detection of various phytoconstituents[13].

Experimental animals

Swiss albino mice weighing 18-25 g of either sex were used for the study. The animals were procured and housed in the animal house maintained under standard hygienic conditions, at 20 ± 2^{o} C, humidity (60 ± 10%) with 12 hour day and night cycle, with food and water *ad libitum*. The study was carried out as per CPCSEA (Committee for the purpose of Control and Supervision of Experiments on Animals) norms after obtaining approval from the Institutional Animal Ethical Committee of MSRCP.

Acute toxicity studies

The acute oral toxicity studies were performed to study the acute toxic effects and to determine minimum lethal dose of the drug extracts. Swiss albino mice of either sex weighing 18-25 g were used for the study. The aqueous and alcohol extracts were administered orally to different groups of over night fasted mice at the doses of 30, 100, 300, 1000 and 3000 mg/kg body weight. After administration of the extracts, animals were observed continuously for the first three hours for any toxic manifestation. Thereafter, observations were made at regular intervals for 24 hrs. Further the animals were under investigation up to a period of one week[14].

Analgesic activity

Analgesic activity of aqueous and alcohol extracts of *Pergularia daemia* (Forsk.) Chiov. was studied by eddy's hot plate and heat conduction method.

Eddy's hot plate method

The animals were divided into six groups of 6 animals each. Group I served as control. Group II served as standard and were injected Diclofenac sodium (9 mg/kg) intraperitonially. Group III and IV were treated orally with aqueous extract of 500 and 1000 mg/kg body weight respectively. Group V and VI were treated orally with alcohol extract of 500 and 1000 mg/kg body weight respectively. The animals were individually placed on the hot plate maintained at 55°C, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds[15].

Heat conduction method

The animals were divided into six groups of 6 animals each. Group I served as control. Group II served as standard and were injected Diclofenac sodium (9 mg/kg) intraperitonially. Group III and IV were treated orally with aqueous extract of 500 and 1000 mg/kg body weight respectively. Group V and VI were treated orally with alcohol extract of 500 and 1000 mg/kg body weight respectively. After one hour, the tip of tail was dipped up to 5 cm into hot water maintained at 58°C. The response time was noted as the sudden withdrawal of the tail from the hot water. Cut off time of 10 seconds was maintained to avoid damage to the tail for all groups. The time required for flicking of the tail, was recorded, to assess response to noxious stimulus[16].

Statistical analysis

All the values ware statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparison test. Comparison between control and drug treated groups were considered to be significant. All values are expressed as mean ± SEM.

RESULTS & DISCUSSION

Pergularia daemia (Forsk.) Chiov. is a drug of good repute in the *Ayurveda* and amongst its multifarious therapeutic uses, mention may be made of its use as analgesic. The roots of *Pergularia daemia* (Forsk.) Chiov. are mentioned as countering poison, anti-inflammatory and analgesic medicine in *Ayurveda*[9].

Acute toxicity studies show that drug is safe up to the dose of 3000 mg/kg with both the extracts. In future it will provide high margin of safety during formulation.

The results of present study indicate the aqueous and alcohol root extracts of *Pergularia daemia* (Forsk.) Chiov. possesses analgesic effect, which is in accordance with its ethnomedical use. Analgesic effect of the extracts was demonstrated in the experimental models using Eddy's hot plate and Heat conduction method using thermal stimuli, an increase in reaction time is generally considered an important parameter of analgesic activity.

The preliminary phytochemical study revealed the presence of alkaloids, carbohydrates, phytosterols, tannins, flavonoids. It helps to undertake further studies on the isolation and identification of

specific phytoconstituents. Both extracts showed the analgesic activity when compared with control and analyzed when analyzed statistically by Tukey Kramer Multiple Comparison Test. On the basis of these findings, it may be inferred that *Pergularia daemia* (Forsk.) Chiov. is an effective agent for analgesic activity. The pharmacological studies carried out with reference to traditional uses of the drug mentioned in *Ayurveda* to justify the claim. In conclusion, this study provides evidences for the analgesic activity of *Pergularia daemia* (Forsk.) Chiov. which could partly contribute to its ethnomedical use.

Table 1: Shows analgesic activity of aqueous and alcohol root extracts of *Pergularia daemia* (Forsk.) Chiov. by Eddy's hot plate method

Groups	Response Time (Mean ± S.E.M.)
Control	2.33 ± 0.2108
Standard (Diclofenac sodium 9 mg/kg)	12.83 ± 0.4014***
Aqueous Extract (500 mg/kg)	4.33 ± 0.3333**
Aqueous Extract (1000 mg/kg)	7.33 ± 0.3333***
Alcohol Extract (500 mg/kg)	6.66 ± 0.3333***
Alcohol Extract (1000 mg/kg)	10.33 ± 0.3333***

One- way Analysis of Variance ANOVA: p value found to be 0.0001 is considered extremely significant. The data were expressed as mean ± S.E.M.; Tukey Kramer multiple comparison test: ***p<0.001, **p < 0.01 (Extracts vs. control)

Table 2: Shows analgesic activity of aqueous and alcohol root extracts of *Pergularia daemia* (Forsk.) Chiov. by heat conduction method

Groups	Response Time (Mean ± S.E.M.)
Control	1.833 ± 0.3073
Standard (Diclofenac sodium 9 mg/kg)	8.5 ± 0.2236***
Aqueous Extract (500 mg/kg)	4.5 ± 0.2236***
Aqueous Extract (1000 mg/kg)	5.5 ± 0.2236***
Alcohol Extract (500 mg/kg)	3.833 ± 0.3073***
Alcohol Extract (1000 mg/kg)	5.833 ± 0.3073***

One- way Analysis of Variance ANOVA: p value found to be 0.0001 is considered extremely significant. The data were expressed as mean ± S.E.M.; Tukey Kramer multiple comparison test: ***p<0.001 (Extracts vs. control)

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