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Original Article

EVALUTION OF ANALGESIC ACTIVITY OF LEAF EXTRACTS OF *PERGULARIA DAEMIA* [FORSK] IN EXPERIMENTAL ANIMALS

NITIN G. SUTAR[1]*, SUBODH C. PAL[2]

[1]Suresh Gyanvihar University, Jaipur, [2]Department of Pharmacognosy, NDMMVP College of Pharmacy, Panchawati Nashik, India. Email: nitingsutar29@yahoo.com

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ABSTRACT

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

Methods: Analgesic activity of petroleum ether and methanol extract of leaf of *Pergularia daemia* [Forsk] was evaluated by using Eddy's hot plate and tail immersion method.

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 extract [300 mg/kg] shows delayed action in paw licking as well as in jumping whereas in the tail immersion method there was delayed time taken for flicking of tail out of water was recorded.

Conclusion: The methanol extract was found to be more potent than petroleum ether extract. The result indicate that the petroleum ether and methanol extract contain such Phytochemical constituents which are responsible for analgesic activity.

Keywords: *Perguleria daemia* [Forsk] Eddy's hot plate, Tail immersion, *Pentazocin.*

INTRODUCTION

Pain is an unpleasant sensory emotional experience associated with actual or potential tissue damage, or described in terms of such damage [1] Although the 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 [2]

Plant introduction

The plant *Pergularia daemia* [Forsk] (Asclepiadaceae) [3] known as "Veliparuthi" in Tamil," Uttaravaruni" in Sanskrit and "Utranjutuka" in Hindi "Uttarni" in Marathi. Traditionally the plant Pergularia daemia is used as anti-helmintic, laxative, antipyretic and expectorant, also used to treat infertile diarrhea and malarial intermittent fevers [4-6]. Latex of this plant used for toothache [7]. Stem bark remedy for cold [8] and fever [9]. Aerial parts of this plant the various pharmacological activities like hepatoprotective [10] antifertility [11] anti diabetic [12] analgesic, anti-pyretic and anti- inflammatory. Phytochemically the plant has been investigated for cardenoloids, alkaloids, and saponins [13]. The plant was found to contain various triterpenes and steroidal compounds [14]. Literature survey of plant shows that the whole plant is useful as analgesic and till so far plant has been no study for analgesic activity. So we have decided to study leaves for analgesic activity.

MATERIAL AND METHODS

Plant Material

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

Extraction procedure

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^{0}C] extraction was continue 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^{0}C] again extraction was continue till a colour 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].

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. Analgesic activity.

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 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 guidelines 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 [30mg/kg], all chemicals of analytical grades *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 the 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. 0.5 gm of extract was dissolved in 5 ml of water then filter it and on filtrate test was performed [15]

Determination of LD_{50} 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_{50} dose, 100mg/kg and 300mg/kg dose were selected and considered as low and high doses respectively. [16]

Assessment of analgesic activity

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 1st (acute model) 11th and 21st day (chronic Model) The temperature of the plate was maintained at 55°C ± 01° C. A cut off reaction time of 30 seconds 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 1st, 11th and 21st day.

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 1st (acute model) 11th and 21st day (chronic Model) The temperature of the water was maintained at 55°C ± 01° C. A cut off reaction time of 30 seconds 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 1st, 11th and 21st day. [17]

RESULTS

Preliminary Phytochemical analysis of Petroleum ether extract of leaf of *Pergularia daemia* [Forsk] shows the presence of Steroids, sterols, tri-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 analgesic

Hot plate method

The results showed that PEPD 300 mg/kg and MPD 300 mg/kg posses significant and equipotent analgesic activity and are less as compared to the reference standard. The potency was found to be same on 11th and 21st day.

Tail immersion method

The results showed that PEPD 300 mg/kg and MPD 100 and 300mg/kg posses significant and analgesic activity. Wherein both the doses of MPD are equipotent and more significant as compared to the PEPD 300 mg/kg dose.

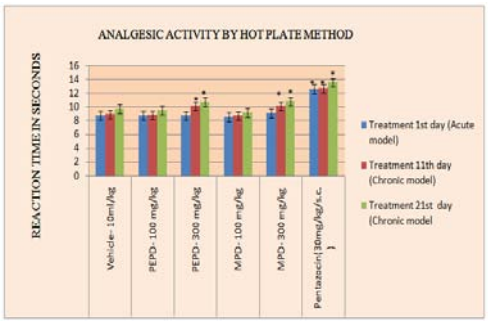

Fig. 1: Effect of Petroleum ether and Methanol extract of leaf of *Pergularia daemia* [Forsk] as analgesic activity by hot plate method

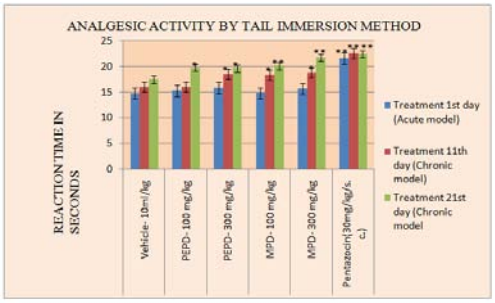

Fig. 2: Effect of Petroleum ether and Methanol extract of leaf of *Pergularia daemia* [Forsk] as analgesic activity by tail immersion method

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 analgesic activity of *Pergularia daemia* [Forsk] may be due to sterols, alkaloid and flavonoids components. The result of present study indicates petroleum ether and methanol leaf extract of *Pergularia daemia* [Forsk] possess analgesic activity which in accordance with ethanomedical use. Analgesic effect of extract was demonstrated in experimental model using eddy's hot plate and tail immersion model. Using thermal stimuli, an increase in reaction time is generally considered an important parameter for analgesic activity. [18] The stimulus may be thermal (tail immersion, and hot plate tests), mechanical (tail or paw pressure tests), electrical (stimulation of paw, tail or dental pulp) or chemical ('writhing' and formalin tests). [19] Acetic acid-induced abdominal constriction is a sensitive method for screening peripheral analgesic effect of compounds. It causes an increase in concentration of PGE2 and PGF2α in the peritoneal fluid.[20,21] The hot plate method and tail immersion method have been found to be suitable for evaluation of centrally acting analgesics.[22] The drug has mixed activity. I. e. both agonist and antagonist actions. Agonist activity is thought to be predominantly at kappa receptors. It acts as a weak antagonist or partial agonist at Mu receptors in the CNS, acting as a CNS depressant The nociceptors seem to be sensitized by sensory nerves. The involvement of endogenous substances such as PGs may be minimized in this model. In centrally acting analgesic methods, the drug in100 mg/kg and 300 mg/kg doses were found to be significantly effective.

CONCLUSION

The methanol extract of leaf of *Pergularia daemia* [Forsk] is more potent than petroleum ether extract of leaf of *Pergularia daemia* [Forsk]. The extract shows dose dependent effect. Further studies are required to find out and isolate active principle and determine the mechanism of action.

CONFLICT OF INTERESTS

Declared None

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