Journal of Comprehensive Pharmacy

Research Article

Available Online at: www.jcponline.in

ISSN NO: 2349-5669

Preclinical evaluation of antinociceptive and anti-inflammatory effects of aqueous plant extract of *Pedaliium murex* Linn.

Sudhakar Pachiappan^{*1}, Vinoth P Veeramani¹, Jothi M Cheenakesavalu², Anand Ramasamy¹

¹ Department of Pharmacology, Swamy Vivekanandha College of Pharmacy, Elayampalayam - 637 205, Tiruchengode, Tamil Nadu, India

² Department of Pharmaceutical Chemistry, Swamy Vivekanandha College of Pharmacy, Elayampalayam - 637 205, Tiruchengode, Tamil Nadu, India

ARTICLE INFO

Article history:

Received 11 January 2016

Accepted 15 February 2016

Available online 28 April

2016

*Corresponding author:

Sudhakar Pachiappan

Email:

sudhakar00pharma@gmail.com

Tel.:+91-9600616196.

ABSTRACT

Aim: The object of the study was to investigate the possible antinociceptive and anti-inflammatory potential of aerial parts aqueous extract of *Pedaliium murex* Linn. (AEPM) in selected experimental animal models.

Materials and Methods: Antinociceptive activity was assessed by acetic acid induced writhings and hot plate analgesic method. Anti-inflammatory effect was evaluated by using carrageenan-induced acute paw edema model.

Results: Primary Phytochemical screening of AEPM indicated the presence of flavonoids, carbohydrate, glycosides, steroids, phenols, alkaloids and tannins. Diclofenac sodium (20 mg/kg p.o.) and codeine (5mg/kg p.o.) was used as reference standard. The AEPM at 200 and 400 mg/kg p.o. showed significant inhibition of abdominal writhing evoked by acetic acid and also increased the pain threshold towards the thermal source in a dose dependent manner. In Carrageenan induced acute rat paw edema the AEPM at a dose of 200 and 400 mg/kg p.o. showed significant ($p < 0.001$) decrease in paw edema volume in a dose dependent manner.

Conclusion: The results of this study concluded that AEPM showed antinociception in acetic acid induced writhing method may be by inhibiting peripheral pain receptor present on cell lining of peritoneal cavity. In hot plate method, may be by involvement of opioid receptor. In the carrageenan induced inflammation AEPM possibly act by inhibiting release and/or action of histamine, serotonin, kinin and prostaglandin like substances.

Key words: *Pedaliium murex* Linn, Phytoconstituents, Antinociceptive, Anti-inflammatory activity.

INTRODUCTION

Medicinal plants have a greater potential to treat chronic and even infectious diseases because they contain a vast array components of therapeutic value. The high degree of efficacy and safety with herbal medicines make them more acceptable compared to other therapeutic invention [1]. Because of lack standardisation and pharmacological screening hinder the potent beneficial action of certain medicinal plants. Among these *Pedaliium murex* Linn. (family: Pedaliaceae) is a diffuse, more or less luscious herb distributed in the coastal area of south india, commonly called as Gokhru [2]. According to Ayurvedic

medicine system gokhru is mainly used as tonic, aphrodisiac, improves appetite and useful in strangury, urinary discharges, vesicular calculi, cough, asthma, pain, cures skin disorders and heart problems, piles and leprosy. It purifies blood, removes stone in bladder. In Unani system it is used as diuretic, gleet, lumbago, tonic, enriches blood, increases mensural flow, good gargles for mouth troubles and painful gums, stomachic, and appetizer [3]. Apart from these also used as analgesic and antipyretic activities [4], an infusion prepared from the *Pedaliium murex* is a highly prized remedy amongst the people of south india in the treatment of gonorrhoea, dysuria and also remedy for spermatorrhoea, incontinence of urine and impotency

[5]. The major vital bioactive phytoconstituents alkaloids, fat, resin, gum, caffeic acid, cumaric acid, daucosterol, ferulic acid, hapatatriacetic acid, vanillic acid, ursolic acid, sitosterol, flavonoids, triterpenoids, steroids, lipids, fatty acids, phenolic acids, amino acids and carbohydrates were reported in various pharmacognostical studies of *Pedaliium murex*. [6, 7, 8]. The present study was focused to evaluating the possible anti-nociceptive and anti-inflammatory effects of aerial parts aqueous extract of *P. murex* Linn.

MATERIALS AND METHODS

Plant collection and identification:

The whole plant of *Pedaliium murex* Linn. Was collected from in and around the area of Namakkal (Tamilnadu) in the south India province of India. Taxonomic identification was confirmed by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Coimbatore, Tamilnadu, India (Ref. No. BSI/SC/5/23/07-08/Tech-1435).

Phytochemical procedure:

Preparation of extract

The aerial part of the plant material includes leaves, stem, flowers and fruits were taken and air dried in shade for ten days. Dried materials were blended into coarse powder and passed through No. 40 sieve and was used for the extraction. The dried coarse powder was extracted by cold maceration process in a narrow mouth bottle for 2 days. After completion of the extraction, it was filtered and dried at 45°C in rotary evaporator to produce a semisolid mass. The dried extract was stored at 4°C until use.

Preliminary phytochemical analysis

The AEPM was subjected to preliminary phytochemical screening through qualitative chemical analysis for confirmation of the phyto constituents [9, 10].

Acute toxicity study:

Albino Wistar rats weighing 180-230 g selected by random sampling were used in this study. Acute oral toxicity was performed as per OECD-423 guidelines [11]. The animals were fasted overnight, provided only with water. The AEPM was administered orally at the dose level of 5mg/kg body weight by gastric intubations and the animals were observed for 14 days. If mortality was observed in 2 or 3 animals, then the dose administered was identified as a toxic dose. If mortality was observed in one animal then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg body weight. The animals were observed for

toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 h.

Animal:

Wistar albino rats (230-250 g) and Swiss albino mice (20-30 g) of either sex were used. The animals were kept under standard environmental conditions (Temperature: $25 \pm 2^{\circ}\text{C}$) and maintained on 12-h light: 12-h dark cycle. They were fed with standard pellet diet and water ad libitum. The animals were acclimatized under laboratory conditions three days prior to initiation of the experiment. The animal care and experimental protocols were in accordance with Institutional Animal Ethical Committee (IAEC) for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) [Reg. No: 889 / ac / 05 / CPCSEA].

Antinociceptive activity:

Antinociceptive activity of the aqueous plant extract of *Pedaliium murex* Linn. Was assessed by acetic acid induced writhing peripheral analgesic method and hot plate central analgesic method in mice.

Acetic acid induced writhing method

Acetic acid induced writhing model was performed by the method of Koster et al., with slight modification [12]. Twenty four albino mice of both sexes were randomly divided into four groups of six mice per group. Group-1 were given 1%CMC solution 10 ml/kg (control group), Group-2 were given Diclofenac sodium 20 mg /kg p.o. (Standard group), while groups 3 and 4 received 200, 400 mg/kg of *Pedaliium murex* Linn. Extract respectively all by gastric gavage. 1 hour after administration of drug and extract, 0.6% glacial acetic acid (10 ml/kg) was given intraperitoneally (i.p) to all the mice to induce pain characterized by abdominal constrictions or writhes. The number of writhes observed in each mouse was counted for 10 minutes and recorded. The percentage protection against abdominal writhing was used to assess the degree of analgesia and was calculated using the formula.

% Inhibition of writhing = $(W_c - W_t/W_c) \times 100$
(Where W_t represents the mean number of writhes in mice treated with test drug and W_c represents the mean number of writhes in control group).

Hot plate method

Hot plate method was performed as described by Eddy and Leimbach [13]. The pre-screened Swiss albino mice showed the reaction time of 3 to 5 sec and were selected and randomly divided into four groups of six mice per group. Group-1 were given 1%CMC solution 10 ml/kg (control group), Group-2 were given codeine 5 mg / kg, p.o. (Standard group), while groups 3 and 4

received 200, 400 mg/kg of *Pedaliium murex* Linn. Extract respectively all by gastric gavage. Animals were placed on the Eddy's hot plate maintained at $55\pm 1^{\circ}\text{C}$. The reaction time in control and treated animals was recorded till the animal showed licking or jumping movements. The cut off time was considered as 10 sec. The reaction time was recorded at 0, 30, 60, 90 and 120 min following administration of the test drug.

Anti-inflammatory activity:

Carrageenan- Induced Paw Edema in Rat

Anti-inflammatory activity of the aqueous plant extract of *Pedaliium murex* Linn. Was assessed by using carrageenan-induced acute paw edema model. The albino Wistar rats of either sex were divided into four groups of six animals each. Group 1 received 1% w/v carboxy methyl cellulose suspension 10 ml/kg orally for 7 days as a control group, Group 2 received Diclofenac sodium 20 mg /kg orally for 7 days, Group 3 and 4 received 200, 400 mg/kg of *Pedaliium murex* Linn. Extract respectively orally for 7 days. Acute inflammation was induced in all groups by injecting 0.1 ml of 1% w/v carrageenan into the sub-plantar region of the right hind paw of rats on 7th day paw volume was measured 1 h prior to carrageenan injection using Plethysmometer and at 0,1,2 and 3 h after the carrageenan injection [14]. Mean increase in the paw volume was measured and percent inhibition was calculated.

% inhibition = $(V_c - V_t/V_c) \times 100$ (Where V_t represents the mean increase in paw volume in rats treated with test drug and V_c represents the mean increase in paw volume in control group).

Statistical analysis:

The data represents as mean $\pm$ SEM of six replicated determinations. Results were analyzed statistically by one way ANOVA followed by post hoc Dunnet's test by using SPSS V.17 (Student trail version). The difference was considered significant when $P < 0.05$.

RESULTS

Phytochemical screening:

The aqueous extract of *Pedaliium murex* were subjected to preliminary phytochemical screening revealed the presence of flavonoids, carbohydrate, glycosides, steroids, phenols, alkaloids and tannins.

Acute oral toxicity study:

The AEPM produced no toxic symptoms or mortality up to a dose level of 2000 mg/kg body weight orally in rats, and hence the drug was considered safe for further pharmacological screening. So 1/10th and 1/5th (200mg and 400mg respectively) of that were selected

for all *in vivo* experiments as sub maximal and maximal dose.

Antinociceptive activity:

Acetic acid induced writhing method

The peripheral analgesic activity of AEPM and Diclofenac sodium on acetic acid induced writhing was shown on Table 1. AEPM and Diclofenac sodium treatment significantly reduced the number of writhes induced by acetic acid in mice. However, effect was higher in Diclofenac sodium treated mice (79.79%) than those treated with *Pedaliium murex* Linn. 200 mg/kg (62.36%) and 400 mg/kg (76.38%) doses.

Hot plate method

The result of the effect of AEPM on the hot plate method is presented in Table 2. The result shows that there was no significant difference in the reaction time during the pre drug testing time. After drug and extract administration, codeine showed the initial response on 30 min. Maximum effect was reached at 90 min. In case of extract early response observed on 60 min in 400 mg/kg treated group ($P < 0.01$), maximal effect at 90 min in 400 mg/kg treated group ($P < 0.01$) and 200 mg/kg treated group ($P < 0.05$).

Anti-inflammatory activity:

Carrageenan- Induced Paw edema in Rat

The anti-inflammatory effect of AEPM and Diclofenac sodium on the Carrageenan induced hind paw edema is presented in Table 3. Extract and Diclofenac sodium produced significant inhibition of paw edema as compared to vehicle control group. Mean percentage of inhibition in AEPM treated groups with 200mg/kg (47.2%) and 400mg/kg (50.0%) doses were comparable to Diclofenac sodium treated group (51.4%) after 3 hr. Anti-inflammatory activity of AEPM was significant ($P < 0.001$) and similar to that of Diclofenac sodium (20 mg /kg) as compared to the vehicle control group.

DISCUSSION

Antinociceptive activities of aqueous extract of *P. murex* Linn. Were evaluated by the acetic acid induced writhing method and hot plate method. These methods allow the analysis of peripheral and centrally mediated antinociceptive responses respectively. Bradykinin, neurokinins, and prostanoids are known mediators for acetic acid-induced writhing [15]. The effect of the AEPM against the noxious stimulus may be it depressed the production of irritants and there by reduction in number of writhes in the mice. The abdominal contraction induced by acetic acid is a sensitive produced to establish peripherally acting antinociceptives. This response is thought to involve local

Table 1: Peripheral analgesic activity of aqueous extract of *P. murex* (Linn.) on acetic acid induced writhing method in mice

Group	Treatment	Total No. of writhing (in 10 min)	% Inhibition
Group-1	Control (1%CMC)	45.17 ± 1.30	-
Group-2	Standard (Diclofenac sodium (20 mg /kg p.o.))	9.13 ± 1.59 ^{***}	79.79%
Group-3	AEPM (200 mg/kg p.o.)	17.00 ± 1.21 ^{***}	62.36%
Group-4	AEPM (400 mg/kg p.o.)	10.67 ± 1.17 ^{***}	76.38%

Values are expressed as Mean ± SEM, n=6, Symbol represents statistical significance: ^{***} P<0.001 One way ANOVA by Dunnet's multiple comparison tests as compared to control.

Table 2: Central Analgesic activity of aqueous extract of *P. murex* (Linn.) on reaction time to hot plate method in mice

Group	Treatment	Reaction time in Sec (Mean ± SEM)				
		Basal	30 min	60 min	90 min	120 min
Group-1	Control (1%CMC)	4.7 ± 0.3	5.0 ± 0.5	5.2 ± 0.3	5.8 ± 0.3	6.2 ± 0.3
Group-2	Standard (codeine (5 mg / kg, p.o.))	4.7 ± 0.3	6.3 ± 0.4 [*]	7.0 ± 0.4 ^{***}	8.0 ± 0.4 ^{***}	7.3 ± 0.3 [*]
Group-3	AEPM (200 mg/kg p.o.)	4.8 ± 0.2	5.3 ± 0.4	6.0 ± 0.2	7.0 ± 0.2 [*]	6.5 ± 0.2
Group-4	AEPM (400 mg/kg p.o.)	4.7 ± 0.1	5.5 ± 0.4	6.5 ± 0.4 ^{**}	7.5 ± 0.4 ^{**}	7.0 ± 0.4 ^{***}

Values are expressed as Mean ± SEM, n=6, Symbol represents statistical significance: ^{***} P<0.001, ^{**} P<0.01 & ^{*} P<0.05 One way ANOVA by Dunnet's multiple comparison tests as compared to control.

Table 3: Anti-inflammatory activity of aqueous extract of *P. murex* (Linn.) on carrageenan induced acute paw edema in wistar albino rats

Group	Treatment	Increase in paw volume in ml (Mean ± SEM)				% edema inhibition after 3 hrs
		0 hrs	1 hr	2 hr	3 hr	
Group-1	Control (1%CMC)	0.33 ± 0.02	0.48 ± 0.03	0.58 ± 0.04	0.72 ± 0.04	-
Group-2	Standard (Diclofenac sodium (20 mg /kg p.o.))	0.35 ± 0.02	0.38 ± 0.03 [*]	0.37 ± 0.02 ^{***}	0.35 ± 0.02 ^{***}	51.4%
Group-3	AEPM (200 mg/kg p.o.)	0.32 ± 0.02	0.43 ± 0.02	0.41 ± 0.04 ^{**}	0.38 ± 0.03 ^{***}	47.2%
Group-4	AEPM (400 mg/kg p.o.)	0.35 ± 0.01	0.40 ± 0.03 [*]	0.38 ± 0.03 ^{***}	0.36 ± 0.04 ^{***}	50.0%

Values are expressed as Mean ± SEM, n=6, Symbol represents statistical significance: ^{***} P<0.001, ^{**} P<0.01 & ^{*} P<0.05 One way ANOVA by Dunnet's multiple comparison tests as compared to control.

peritoneal receptors [16]. The result of the current study indicates that the analgesic effect of *P. murex* Linn. Might be mediated by inhibition the synthesis or acting on peripherally acting nociceptives.

The hot plate method is commonly used to assess the centrally acting analgesics. It measures the complex response to a non-inflammatory, acute nociceptive input and is one of the models normally used for studying central nociceptive activity [17]. It is well established fact that any agent that causes a prolongation of the hot plate latency by this model must be acting centrally [18]. Results of these study indicated that AEPM posses moderate centrally acting analgesic activity in a dose dependent manner on Eddy's hot plate method as compare to the opioid analgesic codeine at 90 min. However, the effect AEPM was higher effect on the acetic acid-induced writhing than on the hot plate method.

Anti-inflammatory activity of *Pedaliium murex* Linn. Was determined by carrageenan induced acute paw edema model, which is one of the most feasible methods to screen anti-inflammatory agents. The development of carrageenan induced oedema is bi-phasic; the first phase is attributed to the release of histamine, serotonin and kinins and the second phase is related to the release of prostaglandins and bradykinins play a major role in the development of inflammatory reaction which is measured at +3 hr [19, 20]. The AEPM produced a significant ($P<0.001$) inhibition of carrageenan induced paw edema at +3h in a dose dependent manner. Therefore, it can be concluded that the inhibitory effect of AEPM on carrageenan induced inflammation could be due to inhibition of the inflammatory enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis. Significant inhibition of paw edema in the early hours after carrageenan injection by AEPM could be attributed to the inhibition of histamine and/or serotonin.

CONCLUSION

In conclusion, results of this study suggest that aqueous extract of aerial parts of *Pedaliium murex* Linn. Has a more predominant peripheral analgesic effect than the central analgesic effect and that the extract is works more significant through the non-opioid mechanism, and also it possesses significant anti-inflammatory effect. However further studies are required for understanding the precise underlying mechanism and characterization of active constituents responsible for Antinociceptive and anti-inflammatory effect.

REFERENCE

1. Chaturvedi A, Kumar MM, Bhawani G, Chaturvedi H, Kumar M, Goel RK. Effect of ethanolic extract of *Eugenia jambolana* seeds

- on gastric ulceration and secretion in rats. *Indian J Physiol Pharmacol.* 2007;**51(2)**:131-140.
2. Nadkarni KM. *Indian Materia Medica*; V. 1, Bombay: Popular Prakashan Private Limited; 1982. P. 229.
3. Patel DK, Laloo D, Kumar R, Hemalatha S. *Pedaliium murex* Linn.: An overview of its phytopharmacological aspect. *Asian Pac J Trop Med.* 2011;**4(9)**:748-755.
4. Shukla VN, Khanuja SPS. Pharmacology and botanical studies on *Pedaliium murex*. *J Med Aromat Plant Sci.* 2004;**26**:64-69.
5. Chopra RN, Nayar SL, Chopra IC. *Glossary of Indian medicinal plants.* 1st ed. New Delhi: National Institute of Science Communication (C.S.I.R); 1956.
6. Das VSR, Rao KN, Rao JVS. Phenolic acid in some members of Pedaliaceae. *Curr Sci.* 1966;**35**:160.
7. Mithal BM and Sagggar SC. Study of *pedaliium murex gum* (Ethoxy gum). *Indian J Pharm.* 1974;**36**:33.
8. Shukla YN, Khanuja SPS. Chemical, pharmacological and botanical studies on *Pedaliium murex*. *J Med Aromat Plant Sci.* 2004;**26(1)**:64-69.
9. Kokate CK, Purohit AP, Gokhale SB. *Textbook of pharmacognosy.* Nirali Prakasan Publication, Pune, India; 2004.
10. Trease GE, Evans WC. *Pharmacognosy.* London, Bailliere Tindall Press; 1983. P.309-706.
11. Ecobichon DJ. *The basis of toxicity testing.* CRC Press, New York; 1997.
12. Koster R, Anderson M, De-Beer EJ. Acetic acid for analgesic screening. *Federation Proceedings.* 1959;**18**:412-418.
13. Eddy NB, Leimbach D. Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. *J Pharmacol Exp Ther.* 1953;**107(3)**:385-393.
14. Venkatesa Perumal R, Adiraj M, Shanmuga Pandiyan P. Synthesis, analgesic and anti-inflammatory evaluation of substituted 4-piperidones. *Indian Drugs.* 2001;**38(3)**:156-159.
15. Ikeda Y, Ueno A, Naraba H, Oh-ishi S. Involvement of vanilloid receptor VR1 and prostanooids in the acid-induced writhing responses of mice. *Life Sci.* 2001;**69(24)**:2911-2919.
16. Chakraborty AR, Devi RK, Rita S, Sharatchandra KH, Singh TI. Preliminary studies on antiinflammatory and analgesic activities of *Spilanthes acmella* in

- experimental animal models. Indian J Pharmacol. 2004;**36(3)**:148-150.
17. Sabina E, Chandel S, Rasool MK. Evaluation of analgesic, antipyretic and ulcerogenic effect of Withaferin A. Int J Integ Bio. 2009;**6(2)**:52-56.
18. Ibiroonke GF, Ajiboye KI. Studies on the anti-inflammatory and analgesic properties of *Chenopodium ambrosioides* leaf extract in rats. Int J Pharm. 2007;**3(1)**:111-115.
19. Brooks PM, Day RO. Nonsteroidal antiinflammatory drugs--Difference and similarities. N Engl J Med. 1991;**324(24)**:1716-1725.
20. Vane J, Botting R. Inflammation and the mechanism of action of anti-inflammatory drugs. FASEB J. 1987;**1(2)**:89-96.

Cite this article as: Pachiappan S, Veeramani VP, Cheenakesavalu JM, Ramasamy A. Preclinical evaluation of antinociceptive and anti-inflammatory effects of aqueous plant extract of *Pedaliium murex* Linn. J Compr Phar 2016;3(2):57-62.