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Antinociceptive activity of aqueous extract of *Pachyptera hymenaea* (DC.) in mice

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Abstract

The standardized aqueous extract of leaves of *Pachyptera hymenaea* (DC.) belonging to family Bignoniaceae was investigated for possible antinociceptive effect in mice. Three different models were used to study the effects of extract on nociception, namely acetic acid-induced writhing test, formalin test (paw licking test) and tail flick test in mice. The extract was administered 1 h prior to pain induction in the dose range of 25, 50 and 75 mg/kg orally. The extract at the given dose range reduced the acetic acid induced nociception by 44.03, 52.90 and 62.46% respectively. The extract reduced formalin effect in both the phases of experiment by 32.36, 41.94, 54.29% and 35.39, 50.17, 55.86% respectively. In the tail flick study, animals' reaction time were increased by 22.69, 38.24 and 40.26% at the above selected doses respectively at 120 min after drug administration. Naloxone (2 mg/kg; s.c.) significantly antagonized the effect of extract in formalin and tail flick method, while partially antagonized the effect in writhing test. However caffeine completely reverted the extract effect in both the phases of formalin test.

Results of these studies revealed that the extract have significant antinociceptive activity in the used models with a possible involvement of central mechanism and adenosine system.

Keywords: Antinociceptive; Formalin test; Paw licking test; *Pachyptera hymenaea*; Tail flick; Writhing test

1. Plant

Pachyptera hymenaea (DC.) belonging to the family Bignoniaceae commonly known as garlic vine (Syn: *Mansao hymenaea*, *Cystista acquinoctalis*, *Pseudocalymma hymenaeum*, *Bignonia hymenaea*) is a woody climber with tendrils on the leaves. The crushed fresh leaves of garlic vine smell similar to that of cloves of *Allium sativum* (garlic) and even used as substitute for garlic in food. The leaves of *Pachyptera hymenaea* (PH) were obtained from medicinal plant garden of our institute and was authenticated at Department of Botany, R.T.M. Nagpur University, Nagpur, India. A specimen was preserved in the college herbarium (JLC/PCG/2006/15).

2. Uses in traditional medicine and other reported activities

Entire plant is used in ayurvedic medicines for treatment of various aliments (Anonymous, 2002). It is used in the treatment of fever, common cold, throat infection, swelling, inflammation and respiratory disorders in Indian and other Asian systems of medicines (Naik, 1998). In South Africa it is traditionally used to treat rheumatoid arthritis and as a muscle relaxant (Luna, 1984). The methanolic extract of PH wood has shown cytotoxic activity against colon cancer cell (Itokawa et al., 1992). Ethanolic and petroleum ether extracts of PH leaves have shown cytotoxic activity against human lung cancer cell line. In the brine shrimp toxicity test, petroleum ether extract had shown higher activity than ethanolic extract. Antioxidant and antimicrobial activities of the petroleum ether extract are higher than those of ethanolic extract (Rugkeart et al., 2005). The dichloromethane and methanolic extracts of leaves have anti-fungal activity (Freixa et al., 1998).

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3. Previously isolated constituents

Pachyptera hymenaea wood contains a napthoquinone compound lapachone (Lawson, 1998).

4. Materials and methods

4.1. Animals

Swiss albino mice (20–25 g) of either sex obtained from animal house of our institute were used. The animals received standard pellet diet (M/s Hindustan Lever Foods, Calcutta, India), water *ad libitum* and were maintained under standard environmental conditions (22° ± 5 °C with 12-h of light/dark cycle). All experimental protocols were approved by the Institutional Animal Ethical Committee (92/1999/CPSEA).

4.2. Chemicals and standard drugs

Pentazocine was obtained from Ranbaxy Laboratories Ltd. (New Delhi, India). Naloxone was obtained from Samarth Pharma Pvt. Ltd. (Mumbai, India). Piroxicam and caffeine were obtained as gift samples from H. Jules and Co. Ltd. (Nagpur, India). Acetic acid, formalin, propylene glycol and tween-80 used were of analytical grade obtained from Loba Chemicals (Mumbai, India). Piroxicam was prepared in required dose (5 mg/kg; i.p.) by dissolving in a mixture of saline, propylene glycol and tween-80 (45:45:10). Pentazocine (2 mg/kg; i.p.) and caffeine (10 mg/kg; i.p.) were prepared in saline solution. The extract was reconstituted in water at the time of oral administration in the doses of 25, 50 and 75 mg/kg body weight.

4.3. Preparation of aqueous extract of PH

The fresh leaves were pulverized and extracted with distilled water by maceration at room temperature for 7 days with occasional shaking. The extract was concentrated to a dark brown colored semisolid mass (yield 17.2%, w/w) under reduced pressure.

4.4. Phytochemical screening

Phytochemical screening of the extract was carried out using conventional protocol (Wagner et al., 1984) for detecting the presence of different constitutes like tannins, saponins, unsaturated sterols, triterpenoids, alkaloids, anthraquinones, flavonoids etc. The saponin content of extract was determined using gravimetric method (Ukpabi and Ukpabi, 2003).

4.5. Acetic acid-induced writhing in mice (Hoessein and Hani, 2002)

Writhing was induced by intraperitoneal injection (0.1 ml/10 g body weight) of 0.6% (v/v) acetic acid. The control, positive control and experimental groups were given the vehicle, control drugs or the extract, one hour prior to the administration of acetic acid. The mice were observed for the number of abdominal constrictions and stretching, counted over a period of 20 min. Naloxone (2 mg/kg; s.c.) was administered 15 min prior to the extract or pentazocine injection to another two groups.

4.6. Formalin test in mice (Tjolsen et al., 1992)

Acute inflammation was induced by planter injection of 25 μl of 5% (v/v) formalin solution (s.c.). The severity of pain response was measured for an hour by scoring method (Vongtau et al., 2004), one hour after the administration of vehicle, control drug and extract. Antinociceptive effect was determined in two phases, the early phase being recorded during the first 5 min while the late phase during last 45 min with 10 min lag period in between the two phases. Nalaxone and caffeine were administered 15 min prior to extract or pentazocine to other groups.

4.7. Tail flick test in mice (Chakraborty et al., 2004)

The tail flick latency was assessed by the analgesiometer (Hicon, India). Basal reaction time was obtained thrice before drug administration and mean (of last two readings) was taken as basal reaction time. Test reaction time was taken at 30, 60 and 120 min after administration of vehicle, control drugs or extract. The cut-off time was fixed at 10 s to avoid tissue damage. If the reading exceeded 10 s, it was considered as maximum analgesia. The opioid involvement was tested by giving nalaxone with the control or extract.

4.8. Acute toxicity studies (Dixon, 1965; OECD, 2000)

Mice were divided into test and control groups ($n = 6$). The test group was given an increasing oral dose (1, 3 and 5 g/kg) of standardized aqueous extract of PH. The mice were allowed food and water *ad libitum* and were kept under regular observation for symptoms of mortality and behavioral changes for the period of 48 h.

4.9. Statistical analysis

The statistical analyses were performed by one-way ANOVA, followed by Dunnett's multiple comparison tests. The results were expressed as the mean ± S.E.M. to show variation in groups. Differences are considered significant when $P \leq 0.05$.

5. Results

5.1. Phytochemical screening

The phytochemical screening of aqueous extract of PH leaves revealed the presence of alkaloids, flavonoids, glycosides, tannins and saponins. Tests for steroids, anthraquinones, lactones/ester and protein/amino acids were found to be negative. The saponin content of the extract was found to be 20% (w/w).

5.2. Acute toxicity studies

There was no mortality at doses up to 5 mg/kg (p.o.) in mice. During observation the animals exhibited decreased mobility but no signs of convulsions or loss of writhing reflex. This result indicates PH leaves extract has low toxicity profile.

5.3. Antinociceptive activity

The aqueous extract of PH (25, 50 and 75 mg/kg; p.o.) showed significant antinociceptive activity in all the selected models. The extract decreased the number of acetic acid-induced abdominal constrictions in mice and the values were found to be significant ($P < 0.01$) at dose levels tested. The maximum percentage inhibition of constrictions (62.46%) was observed at 75 mg/kg for the extract, which was statistically similar to the control drug piroxicam and slightly lower than pentazocine. This effect was partially antagonized by pretreatment with naloxone, an opioid receptor antagonist (Fig. 1).

The formalin test in mice revealed a similar pattern of antinociceptive effect (Fig. 1). There was a significant reduction in responses to nociception during the early and late phase with the extract at 75 mg/kg, when compared to the vehi-