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Antinociceptive and hypnotic effects of *Premna tomentosa* L. (Verbenaceae) in experimental animals

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Abstract

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folklore use as pain relievers should therefore be viewed as a fruitful and logical research strategy in the search of new analgesic drugs. In the present inquiry, antinociceptive effects of *Premna tomentosa* (PT) leaf extract (in methanol) were explored in experimental animals by acetic acid-induced writhing, tail flick and tail clip tests. Oral administration of PT extract at different doses (100, 200, 400 and 500 mg/kg) led to significant antinociceptive effects. The extract was also tested for hypnotic effects. Treatment with extracts at different doses (100, 200, 400 and 500 mg/kg) decreased the locomotor activity and potentiated the pentobarbitone-induced sleep time. The responses were dose-dependent. On the basis of the present finding, we can conclude that PT possesses antinociceptive and hypnotic activities.

Keywords: *Premna tomentosa*; Antinociceptive; Hypnotic; CNS-depressant

1. Introduction

Drugs of natural origin continue to be important for the treatment of many diseases worldwide. *Premna tomentosa* (PT) L. (Verbenaceae) commonly called as "Krishnapalai" or "Pudangai Nari" is a medicinal plant used extensively for the treatment of various disorders. It is a moderately sized deciduous tree with shoots, leaves and inflorescence densely clothed with a tawny yellow stellate tomentum. The bark is light grayish brown, like that of teak and flowers are greenish yellow (Haines, 1961). Indian Siddha medicine claims a lasting cure for hepatic disorders through oral administration of aqueous extract of PT (Shanmugavelu, 1987). In traditional medicine, PT has also been used to treat stomach disorders and diarrhea. The leaves also possess diuretic properties (Anonymous, 1989). Earlier studies have shown that the methanolic extract of PT leaves afforded protection against acetaminophen-induced hepatotoxicity in rats (Devi and Devaki, 1998) by its antioxidant property (Devi et al., 1998). Preliminary phytochemical screening of the leaves revealed the presence of 57.8% D- and DL-limonene, 17.2% β-caryophyllene, 7.8% cadalene-type sesquiterpene, 5.6% sesquiterpene tertiary alcohol and 5.5% aditerpene (Lakshminarayen and Muthana, 1953).

Concerning the pharmacological properties of PT, only the anti-inflammatory effect (prevention of the cotton pellet granuloma) of the plant is reported (Alam et al., 1993). To further explore the pharmacological properties of the plant extract, we have investigated the plant extract for its antinociceptive and hypnotic effects.

2. Methods

2.1. Plant extract

PT leaves were collected from a rural area near Palayamkottai, Tamil Nadu, India and authenticated by Dr. Usman Ali, Drug Research Scheme, Multidisciplinary Unit, Central Research Institute for Siddha, Chennai-106,

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India. The powdered leaves were extracted with methanol to a syrupy mass. The last traces of the solvent were removed in vacuum with an approximate yield of 12.4%.

2.2. Animals

Male Wistar albino rats (150–200 g) and Laka albino mice (20–30 g) were obtained from the Fredrick Institute for Plant Protection and Toxicology, Padappai, Chennai, India. The animals were acclimatized to laboratory conditions for 10 days during which they were fed a commercial pellet diet (M/s Hindustan Foods, Bangalore, India) and water ad libitum. The animal experiments (six in each group) were performed according to internationally followed ethical standards and approved by the research ethics committee of University of Madras.

2.3. Assessment of antinociceptive effect of PT extract

2.3.1. Acetic acid-induced writhing assay

Analgesic activity of the plant extract was studied by reduction of acetic acid-induced writhing in mice (Koster et al., 1959). Thirty minutes after the administration of the plant extracts (100, 200, 400 and 500 mg/kg, orally) or standard aspirin (100 mg/kg, orally), the animals received acetic acid (0.6%, 10 ml/kg ip). The number of abdominal contractions (writhings) and stretchings with a jerk of the hind limb was counted for 15 min after administering acetic acid, and percent inhibition was calculated as follows

$$\% \text{ Inhibition} = (1 - W_T/W_C) \times 100$$

where W_T is the writhings in drug-treated mice and W_C is the writhings in control mice.

2.3.2. Tail flick test

The tail flick response of rats was measured by means of tail flick unit. The tail of the rats was placed on a hot wire, and the time taken by the animal to withdraw (flick) its tail from the hot wire was taken as the reaction time (D'Amour and Smith, 1941). A cutoff time of 20 s was followed to prevent any injury to the tail. The tail flick test was performed after the oral administration of the plant extract (100, 200, 400 and 500 mg/kg) or the reference drug aspirin (100 mg/kg, orally) and the mean reaction time was noted.

2.3.3. Tail clip test

Albino mice used for the experiment were screened by applying a metal artery clip to the base of the tail with its jaws sheathed with thin rubber tubing. The pressure exerted by the clip was so adjusted that it was just sufficient to make all control mice respond. Those animals that did not show efforts to dislodge the clip within 15 s were not used for the experiment (Takagi et al., 1966). Mice showing a positive response were given olive oil (0.1 ml, orally), reference drug aspirin (100 mg/kg, orally) or different doses of the plant extract (100, 200, 400 and 500 mg/kg, orally). The tail clip was applied after 30 min and the mean reaction time was noted.

2.4. Potentiation of pentobarbitone-induced hypnosis in mice

The sleeping time in mice was studied by the method of Dandiya and Collumbine (1959). The mice received chlorpromazine (10 mg/kg ip) as standard CNS depressant, ephedrine hydrochloride (10 mg/kg ip) as standard stimulant or different doses of the plant extract (100, 200, 400 and 500 mg/kg, orally). After 30 min, sodium pentobarbitone (60 mg/kg ip) was administered and the sleep time was recorded.

2.5. Locomotor activity in mice

Locomotor activity (ambulatory score) was measured using an animal activity meter (Columbus Instruments, OH, USA). An array of 15 infrared emitter/detector (spaced at 2.65 cm intervals; beam wavelengh = 87 nm; distance between the sensors = 50 cm) measured the animal activity along a single axis of motion, the digital data being displayed on the front panel meters as ambulatory activity. The locomotion was expressed in terms of total photobeam counts per 5 min. The animals were allowed to adapt to the new environment for at least 5 min and then the locomotor activity was counted. The plant extract (100, 200, 400 and 500 mg/kg, orally) or the standard drug (chlorpromazine and ephedrine hydrochloride at 10 mg/kg as standard depressant and stimulant, respectively) was administered 30 min before the assessment of locomotor activity.

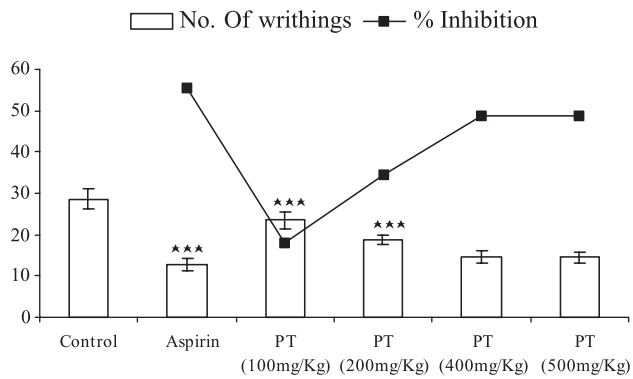

Fig. 1. Antinociceptive response of PT extract by acetic acid-induced writhing in mice. Values are expressed as mean ± S.D. ($n = 6$). Statistical comparisons: *** $P < .01$ for aspirin versus control and PT (100 and 200 mg /kg) versus aspirin.

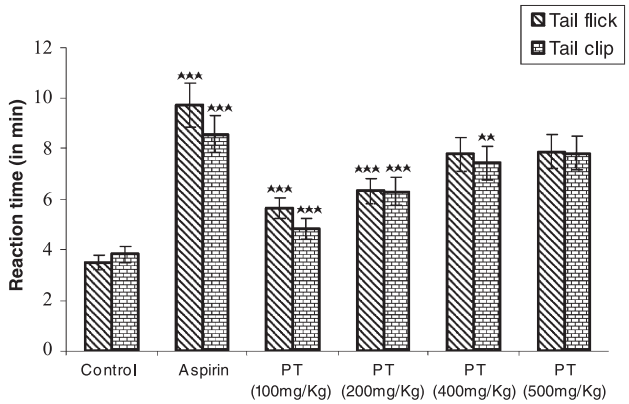

Fig. 2. Antinociceptive response of PT extract (by tail flick and tail clip methods) in rats. Values are expressed as Mean ± S.D. ($n = 6$). Statistical comparisons: *** $P < .01$ for aspirin versus control; *** $P < .01$ for PT (100 and 200 mg/kg) versus aspirin (both tail flick and tail clip tests); ** $P < .05$ for PT 400 mg/kg versus aspirin (tail clip test).

2.6. Statistical analysis

The results were expressed as mean ± S.D. All statistical comparisons were made by Dunnetts test after conducting one-way ANOVA.

3. Results

3.1. Effect of the extract to relieve pain

The oral administration of PT extract (both at 400 and 500 mg/kg) caused 48% inhibition of the writhing numbers of mice induced by acetic acid. Doses at 100 and 200 mg/kg showed no significant reduction when compared to aspirin-treated animals, whereas significant reduction ($P < .01$, F value = 64.77) was observed when compared to vehicle-treated control group (Fig. 1). The extract produced a dose-dependent decrease in writhing and an increase in pain threshold in tail clip test (Fig. 2). In tail flick test, no significant difference was observed between animals treated with 100 and 200 mg/kg of extract, but significant ($P < .01$, F value = 55.58) difference was seen between 200 and 400 mg/kg treated animals and 200 and 500 mg/kg treated animals (Fig. 2). A significant ($P < .01$, F value = 48.95) resistance to pain threshold was exerted at both the concentrations 400 and 500 mg/kg of PT extract in tail clip test when compared to aspirin-treated group. Whereas, the pain threshold resistance was only moderate at both the doses 400 and 500 mg/kg of PT extract when assessed by tail flick test.

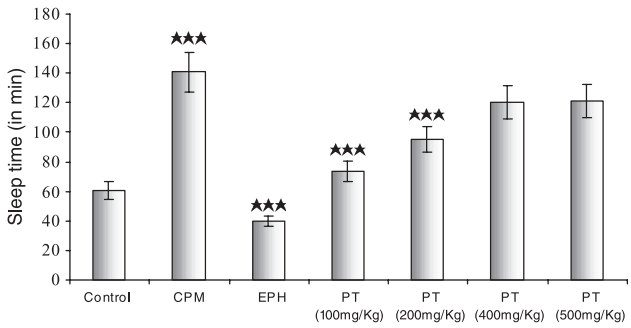

Fig. 3. Effect of PT extract on pentobarbitone-induced sleep time in mice. Values are expressed as Mean ± S.D. ($n = 6$). Statistical comparisons: *** $P < .01$ for CPM/EPH versus control and PT (100 and 200 mg/kg) versus CPM. CPM—Chlorpromazine, EPH—Ephedrine hydrochloride.

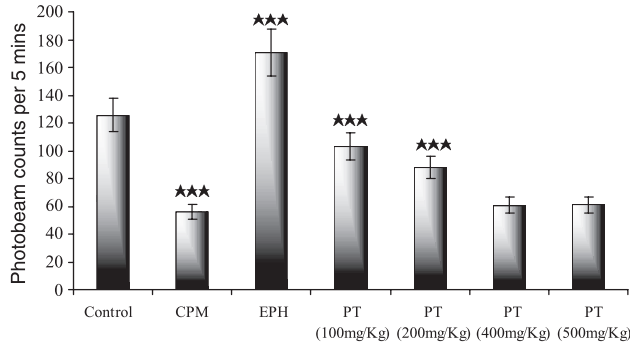

Fig. 4. Effect of PT extract on locomotor activity (by photoactometer) in rats. Values are expressed as Mean ± S.D. ($n = 6$). Statistical comparisons: *** $P < .01$ for CPM/EPH versus control and PT (100 and 200 mg/kg) versus CPM. CPM—Chlorpromazine, EPH—Ephedrine hydrochloride.

3.2. Effect of the extract on pentobarbitone-induced hypnosis and locomotion

Oral administration of PT extract offered a moderate increase in sleep time after doses 400 and 500 mg/kg when compared to the standard depressant chlorpromazine (Fig. 3). In locomotor activity, there was no significant difference between the animals treated with 100 and 200 mg/kg of PT extract, whereas significant difference ($P < .01$, F value = 93.2) was observed between groups treated with doses 200 and 400 mg/kg of extract (Fig. 4). The decrease in locomotor activity was significantly ($P < .01$, F value = 93.2) comparable to the standard depressant chlorpromazine at both doses 400 and 500 mg/kg.

4. Discussion

Current analgesia-inducing drugs such as opiates and NSAIDs are not useful in all cases because of their side effects and potency. As a result, the search for other alternatives seems necessary and beneficial. In this process, animal studies are necessary to provide objective evaluation of the efficacy of analgesic drugs, which are a requirement for the registration of a new drug. Hence, the present investigation was carried out in experimental animals treated with PT extract. The results of the present study

revealed that PT extract has a potent antinociceptive activity in experimental animals. The extract produced a dose-dependent increase in the pain threshold in acetic acid-induced nociception as the number of writhes decreased during 15 min as compared to the control group. Acetic acid-induced writhing is related to the increase in the peritoneal fluid levels of PGE_2 and $PGF_{2\alpha}$ (Deraedt et al., 1980) and the test can detect antinociceptive compounds that may be inactive in the tail flick assay (Bentley et al., 1983). The abdominal-constriction response is thought to involve, in part, local peritoneal receptors, while the tail flick response is predominantly a spinal reflex (Jais et al., 1997). It is therefore possible that PT extract exerts an analgesic effect probably by inhibiting synthesis or action of prostaglandins.

In the tail flick assay, which uses a thermal stimulus and which is considered as an important parameter of central analgesic activity (Beirth et al., 1998), the extract exhibited an increase in reaction time. The extract also produced a dose-dependent increase in reaction time in tail clip method. From the results, it is clear that the methanolic extract of PT offers protection to the animals against pain.

Pentobarbitone-induced sleeping time and locomotor activity (by photoactometer) were performed to detect the activity of the plant extract on the central nervous system. The extract prolonged pentobarbitone-induced hypnosis dose dependently. The decrease in sleep latency and increase in sleeping time are classically related to the central nervous system depressant drugs (Sharpley et al., 1996). The depressant activity of the extract may also be attributed to the inhibition of pentobarbitone metabolism or to an action on the central mechanism involved in the regulation of sleep (Kaul and Kulkarni, 1978). The decrease in locomotor activity further confirms the depressant activity.

On the basis of the present study, we may conclude that the methanolic extract of PT has antinociceptive and hypnotic effects. The sedative and analgesic effects of PT leaves may be attributed to the presence of limonene and caryophyllene in the leaves, since it is reported in Dr. Dukes Phytochemical and Ethnobotanical Databases (USDA-ARS-NGRI, Beltsville Agricultural Research Centre, Maryland) that limonene possesses sedative property and caryophylene possess anti acne and sedative property.

Acknowledgements

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