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Antipyretic, antinociceptive and anti-inflammatory activity of *Premna herbacea* roots

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Abstract

The alcoholic extract of the roots of *Premna herbacea* was investigated for its antipyretic, antinociceptive and anti-inflammatory potential in animal models. The extract, when administered orally to mice has been found to be safe up to a dose of 8.0 g/kg. A significant antipyretic effect has been observed in rabbits while mild antinociceptive effects were evidenced in mice when tested by chemical as well as thermal methods. The extract did not exhibit any anti-inflammatory activity in acute but significantly reduced the chronic inflammation.

Keywords: Premna herbacea; Antipyretic activity; Antinociceptive activity; Anti-inflammatory activity

1. Introduction

Premna herbacea Roxb., syn. *Pygmacopremna herbacea* (Roxb.) Mold. (Verbenaceae), known as *Sirutekku* in Tamil, is used in *Siddha*, the traditional

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system of medicine practised in south India. *Sirutekku* is claimed to be useful in treating fevers (including malarial fever), inflammation, rheumatism, respiratory disorders and as a sedative [1,2]. As there is no scientific evidence in support of these claims, the roots of this plant were subjected to pharmacological screening.

2. Experimental

2.1. Plant material

P. herbacea roots, collected in October 1996, procured from Thiruvananthapuram crude drug market (Kerala State) and identified by Dr V. Chelladurai, Survey of Medicinal Plants Unit (CCRAS, Govt of India), Palayamkottai, Tamil Nadu. A voucher specimen (Pharm No.15/96) has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Madras Medical College, Chennai.

2.2. Preparation of extract

Shade dried and powdered root (2.8 kg) was extracted with EtOH at room temperature (72 h) and the extract was concentrated to obtain a semisolid residue (yield: 7.07%). Phytochemical screening [3] gave a positive test for phenolics.

2.3. Animals

Swiss albino mice (20–25 g), Wistar albino rats (150–200 g) and New Zealand rabbits (1.5–2.0 kg) of either sex maintained in the Animal Experimental Laboratory of Madras Medical College, under standard animal housing conditions (temperature 25 $\pm$ 2°C, relative humidity 75 $\pm$ 5% and 12-h light and dark cycle) were used for experiments. The animals had access to standard laboratory feed (M/s. Hindustan Lever Ltd.) and water ad libitum.

2.4. Acute toxicity

Mice were divided into five groups, each containing six animals. *P. herbacea* extract was administered orally at doses ranging from 0.5 to 8.0 g/kg following a standard method [4]. Animals were continuously observed for 2 h to detect changes in the autonomic or behavioural responses and then monitored for any mortality for the following 7 days. A group of animals treated with the vehicle (0.5% carboxy methyl cellulose sodium served as control. Based on the results of preliminary toxicity testing, the doses of 100, 200 and 400 mg/kg were chosen for further experiments.

2.5. Antipyretic activity

The method of Brownlee [5] was used. Rabbits maintained in the laboratory for

24 h prior to the experiment were used. Typhoid-Paratyphoid A,B (TAB) vaccine (0.1 ml) was injected into the marginal ear-vein and the rectal temperature was recorded every 15 min using 'Electrolab' 18 channel telethermometer. *P. herbacea* extract was administered orally in doses of 100, 200 or 400 mg/kg, 60 min after TAB vaccine injection. Subsequently, the rectal temperature was recorded every 30 min up to 3 h. Paracetamol (100 mg/kg, p.o) was used for comparison.

2.6. Antinociceptive activity

2.6.1. Acetic acid-induced writhing

The method of Koster et al. [6] was used. Three different groups of six mice each received the test extract at 100, 200 or 400 mg/kg of *P. herbacea*. Sixty minutes later 0.6% acetic acid (10 ml/kg) was intraperitoneally injected. The number of writhings during the following 15-min period were counted. A significant reduction in the number of writhings compared to the control animals was considered as an antinociceptive response. Morphine sulphate (5 mg/kg, s.c.) was used for comparison.

2.6.2. Hot plate test

The method of Eddy and Leimbach [7] was used. Mice were treated orally with different doses (100, 200 or 400 mg/kg) of test extract. The animals were placed on a hot plate maintained at 55 $\pm$ 0.5°C. The time taken to lick the hind-paw (taken as the reaction time) was measured just prior to drug administration and then at 30-min intervals up to 3 h. For each animal, reaction times were plotted against the time of observation and the area under the time response curve was calculated as a measure of antinociceptive activity. Morphine sulphate (5 mg/kg, s.c.) was used for comparison.

2.7. Anti-inflammatory activity

2.7.1. Acute inflammation

The method of Winter et al. [8] was used (carrageenin-induced paw-edema in rats). One percent carrageenin suspension (0.1 ml) was injected into the plantar surface of the right hind-paw. The extract (100, 200 or 400 mg/kg.) was administered orally 60 min prior to carrageenin injection. Control animals received an equal volume (10 ml/kg) of vehicle while phenylbutazone (100 mg/kg, p.o) was used as the standard drug. The paw volume was measured 4 h later using a plethysmograph. The difference in volume between the left and the right hind-paws was taken as a measure of edema.

2.7.2. Chronic inflammation

The method of Winter and Porter [9] was used (cotton-pellet granuloma in rats). Under light ether-anaesthesia, sterile cotton-pellets weighing 10 mg were implanted subcutaneously in the arm-pits. The animals were treated orally with

different doses of extract (100, 200 or 400 mg/kg/day) for 7 days. They were killed on the 8th day, the cotton pellets were removed, freed from adhering tissue, dried at 50°C for 24 h and weighed. Control and standard drug (phenylbutazone) groups were used for comparison as in the acute model.

2.8. Statistical analysis

Differences between control and treated groups were tested for statistical significance by the Student's *t*-test.

3. Results

3.1. Acute toxicity

The alcoholic extract of *P. herbacea* roots, when orally administered in the dose range of 0.5–8.0 g/kg to mice, did not produce any significant change in the autonomic or behavioural responses during the observation period. No mortality was observed up to the 7th day of monitoring.

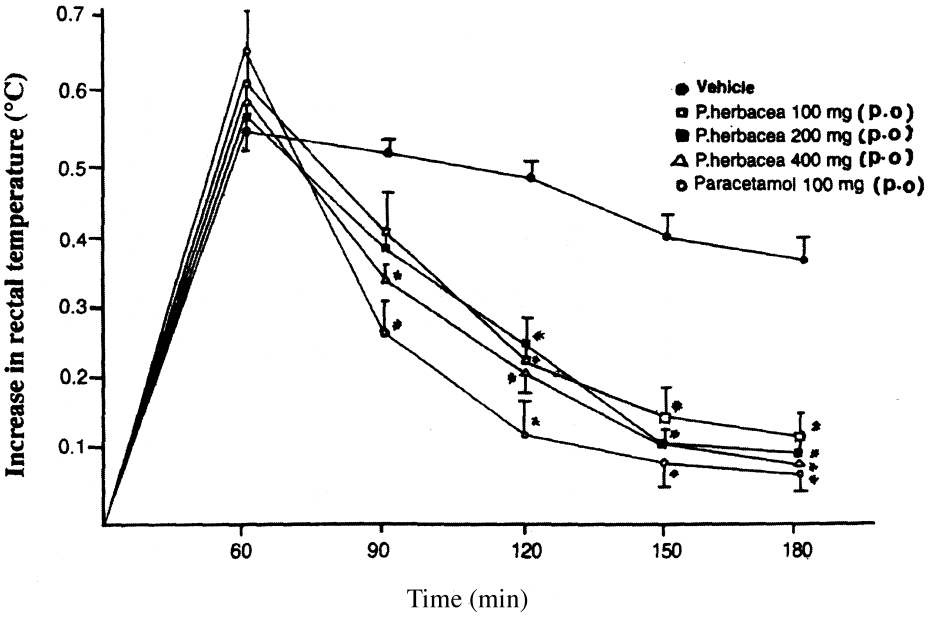

Fig. 1. Effect of ethanol extract of *P. herbacea* roots on TAB vaccine-induced pyrexia in rabbits. Results are mean ± S.E.M., $n = 6$; $^*P < 0.01$ vs. control, Student's *t*-test.

Table 1
Effect of the ethanol extract of *P. herbacea* roots on acetic acid-induced writhing and hot plate methods in mice[a]

Treatment (route)	Dose (mg/kg)	Acetic acid-induced writhing Number of writhings	Hot plate AUC (cm^2)
Control (vehicle, 10 ml/kg, p.o)[b]	–	30.3 ± 0.90	1.63 ± 0.43
Morphine sulphate (s.c.)	5	1.0 ± 0.20**	32.60 ± 1.10**
P. herbacea (p.o.)	100	26.0 ± 1.65*	5.90 ± 0.62**
	200	24.2 ± 1.80*	7.10 ± 1.40**
	400	23.5 ± 1.70*	7.70 ± 1.58**

[a] Values are mean ± S.E.M., $n = 6$; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control; Student's *t*-test.
[b] Vehicle: 0.5% carboxy methyl cellulose sodium suspension.

3.2. Antipyretic activity

Administration of TAB vaccine in rabbits produced a significant increase in rectal temperature at 60 min, which gradually decreased after 120 min. Treatment with *P. herbacea* (100–400 mg/kg, p.o) significantly reduced the rectal temperature, dose dependently during the whole period of observation (Fig. 1).

3.3. Antinociceptive activity

Morphine treatment significantly reduced the number of acetic acid-induced writhings and also showed a significant increase in the AUC in hot plate method. Following *P. herbacea* (100–400 mg/kg, p.o.) significant but not dose-dependent effects were observed with both methods (Table 1).

3.4. Anti-inflammatory activity

In carrageenin-induced paw edema method (acute model), the standard anti-inflammatory agent phenylbutazone produced a significant reduction in the volume of paw edema whereas no reduction was observed with *P. herbacea* extract. On the contrary, a mild, but statistically significant reduction was observed in the weight of cotton pellets in animals treated with 200 and 400 mg/kg of *P. herbacea* (Table 2).

4. Discussion

Among several traditional claims, the effectiveness of *Sirutekku* in fever and inflammation has been particularly emphasised [2]. Hence, it was considered that pharmacological investigation on *P. herbacea* for these properties was deserved to scientifically validate the traditional claims. The antinociceptive effect was also

Table 2
Effect of the ethanol extract of *P. herbacea* roots on carrageenin-induced paw edema and cotton pellet granuloma methods in rats[a]

Treatment (route)	Dose (mg/kg)	Carrageenin-induced edema Paw volume (ml)	Cotton pellet granuloma Pellet weight (mg)
Control (vehicle, 10 ml/kg, p.o.)[b]	–	0.75 ± 0.07	50.18 ± 1.05
Phenylbutazone (p.o)	100	0.28 ± 1.10**	28.25 ± 1.30**
P. herbacea (p.o.)	100	0.80 ± 0.06	49.08 ± 2.04
	200	0.80 ± 0.00	44.31 ± 1.92*
	400	0.73 ± 0.11	43.81 ± 1.41**

[a] Values are mean ± S.E.M., $n = 6$; *$P < 0.05$, **$P < 0.01$ vs. control; Student's t-test.
[b] Vehicle: 0.5% carboxy methyl cellulose sodium suspension.

investigated since most of the non-steroidal agents used against fever and inflammation also possess antinociceptive activity.

The results of acute toxicity testing indicate that the ethanol extract of *P. herbacea* is safe even up to an oral dose of 8.0 g/kg.

The reduction in pyrexia after *P. herbacea* administration indicates the antipyretic effect of this plant, the response at higher doses being almost comparable to that of paracetamol.

The results of acetic acid-induced writhing and hot plate tests indicate a mild antinociceptive activity for *P. herbacea*.

P. herbacea seems to be devoid of any effect on inflammation after single dose administration, as the volume of paw edema was not significantly reduced when compared to vehicle treatment. However, the results of cotton pellet granuloma reveal a significant anti-inflammatory effect after repeated administration.

Thus, the results of the present study provide support to the traditional usage of *P. herbacea* in fever and less prominently in nociception and inflammation, even if further studies are needed to better evaluate these activities and the potential of the plant.

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References

[1] Nadkarni KM. Indian materia medica. Bombay, India: Popular Prakashan Pvt. Ltd, 1954:1009.
[2] Murugesa Mudaliar KS. Materia medica, Part 1. 3rd ed. Govt. of Tamil Nadu, 1969:169.
[3] Harborne JB. Biochemistry of phenolic compounds. London: Academic Press, 1968:35.

[4] Turner RA. Screening methods in pharmacology, vol. 1. New York: Academic Press, 1965:27.
[5] Brownlee G. J Pharm Pharmacol 1937;10:609.
[6] Koster RM, Anderson M, DeBeer EJ. Fed Proc 1959;18:412.
[7] Eddy NB, Leimbach D. J Pharmacol Exp Ther 1953;107:385.
[8] Winter CA, Risley EA, Nuss GW. Proc Soc Exp Biol Med 1962;111:544.
[9] Winter CA, Porter CC. J Am Pharm Assoc 1957;46:515.