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Assessment of neuropharmacological activities of *Pandanus foetidus* (Pandanaceae) in mice

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Received May 9, 2005, accepted June 15, 2005

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Pharmazie 61: 362–364 (2006)

The methanol extract of the leaves of *Pandanus foetidus* Roxb. (Pandanaceae) was assessed for neuropharmacological activities in mice using a number of experimental models. The extract dose-dependently inhibited acetic acid-induced writhing in mice when given at the doses of 250 and 500 mg/kg body weight. At the same dose levels, it significantly prolonged the pentobarbitone-induced sleeping time in mice, and showed mild to moderate central nervous system depressant activity when assessed by the hole cross and the open field tests in mice model. On the basis of these findings, it can be assumed that the extract exerts its depressant effect on the central nervous system in mice by interfering with the cortical function.

1. Introduction

Pandanus foetidus Roxb. (Pandanaceae), 'kewa kata', a common hedge-plant with no proper stem, grows throughout Bangladesh, mainly in the coastal region of the mangrove forest, Sundarban (Chaffey and Sandom 1985; Prain 1981). Leaves of this plant are used in leprosy, small pox, syphilis, scabies, and heart and brain diseases (Joshi 2000; Yusuf et al. 1994). Leaves and spadix are also used in diabetes (Yusuf et al. 1994). *P. foetidus* yields essential oils for perfumery as well as medicinal uses. As part of our on-going pharmacological studies on selected medicinal plants from the Sundarban mangrove (Uddin et al. 2004, 2005), we now report on the neuropharmacological effects of the extract of *P. foetidus* in mice using various established experimental models.

2. Investigations, results and discussion

In the acetic acid induced writhing assay (Koster et al. 1959), the methanol (MeOH) extract of *P. foetidus* leaves displayed dose-dependent inhibition of acetic acid-induced writhing in mice. At the doses of 250 and 500 mg/kg body weight, the extract produced, respectively, 53.95 and 69.93% inhibition of acetic acid-induced writhing in the test animals (Table 1), which was statistically significant ($P < 0.001$). Salicylates and similar analgesic drugs can prevent the writhing response in rodents induced by intraperitonial (i.p.) administration of dilute acetic acid, phenylquinone or bradykinin. However, several other compounds without having any analgesic property can also prevent the writhing response, and that is why this test may not be entirely specific. Nevertheless, when carried out in conjunction with other tests, including the ability to inhibit prostaglandin (PG) synthesis, especially that from nervous tissue, the anti-writhing test, can provide useful pharmacological information (Bowman and Rand 1980).

In vivo methods using intact animals are considered to be the best method for investigating the action of drugs on the central nervous system (CNS). The most important step in evaluating drug action on the CNS is to observe the behaviour of the test animals. To obtain meaningful results regarding the effect of *P. foetidus* extract on the CNS in mice, a number of methods namely pentobarbitone-induced hypnosis, the open field and the hole cross tests were adopted (Gupta et al. 1971; Takagi et al. 1971).

Table 1: Effect of *P. foetidus* Roxb. leaf extract on acetic acid induced writhing in mice

Treatment	Dose[a] (mg/kg, p.o.)	Writhings[b]	Inhibition (%)
Control (1% Tween 80, 0.01ml/gm, p.o.)	–	30.60 ± 1.04	–
Aspirin	50	5.0 ± 0.79[a]	83.66
P. foetidus Roxb. leaf extract	250	14.20 ± 0.56[a]	53.95
	500	9.20 ± 1.39[a]	69.93

[a] Administered 30 min before 0.7% acetic acid administration (0.01 ml/g, i.p.)
[b] Counted for 15 min, starting 5 min after acetic acid administration; values are mean ±S.E.M
[a] $P < 0.001$ vs. control, Student's t-test; $n = 5$

Table 2: Effect of *P. foetidus* leaf extract on pentobarbitone-induced sleeping time in mice[a]

Treatment	Dose (mg/kg)	Latent period (min)	Duration of sleep (min)
Control (1% Tween 80 in water)	10 ml/kg	7.2 ± 0.96	65.6 ± 4.85
Diazepam	1	3.4 ± 0.570[b]	90.8 ± 2.90[b]
P. foetidus	250	5.40 ± 0.570	73.4 ± 4.8
	500	4.0 ± 0.79[c]	87.4 ± 3.99[b]

[a] Values are mean ± S.E.M. (n = 5)
[b] $P < 0.01$, [c] $P < 0.05$ vs control, Student's t-test

In the pentobarbitone-induced hypnosis, *P. foetidus* extract, at the doses of 250 and 500 mg/kg, induced sleep at a rapid stage as compared to control, and increased the duration of sleep, which might be attributed to the action on the cerebral mechanism involved in the regulation of the sleep. The extract reduced the latent period for the onset of sleep, and increased the total sleeping time at both dose levels, but statistically significant results were obtained only at the higher dose ($P < 0.05$ for the onset of sleep and $P < 0.01$ for the total sleeping time) (Table 2).

In the hole cross test (Takagi et al. 1971), the extract given in the same doses, caused a mild to moderate depressant effect on the CNS. The statistically significant peak effect was observed, at both dose levels, at the second observation period (30 min) after administration of the extract, and continued to the last observation period (240 min) (Table 3).

In the open field test (Gupta et al. 1971), the result of the second observation period (30 min) showed a noticeable decrease in locomotion in the test animals by the extracts. As time went by, the depressant effect became more intense and persisted throughout the entire observation period with little variation. The results were statistically significant (Table 4).

In summary, the data obtained from several neuromorphological experiments, demonstrated that *P. foetidus* leaf extract possesses CNS depressant activity as evident from the reduced explorating activity of mice in the open field test, reduced movement in the hole cross test, and prolonged sleeping time in the pentobarbitone-induced sleeping time test. Though the mechanism of this depression is not clearly understood at this stage, it can be assumed that the extract might have exerted its central depressant effect by interfering with the function of the cortex.

3. Experimental

3.1. Plant material

The leaves of *P. foetidus* Roxb. were collected from the Sundarban mangrove forest, Khulna, Bangladesh and was authenticated by Professor Abdul Matin, Forestry Discipline, Khulna University, Khulna. The leaves of the plant were collected and shade dried and finally pulverised into coarse powder.

3.2. Extraction

The dried and powdered leaves *P. foetidus* (560 g) were subjected to maceration by MeOH (800 mL) at r.t. for 2 days. The resulting extract was filtered, and the solvent was evaporated using rotary evaporator (50 °C).

3.3. Animals

Swiss albino mice of either sex (20–25 g) were obtained from the Animal House, Pharmacy Discipline, Khulna University, Khulna. The animals were housed under standard laboratory conditions (relative humidity 55–65%, r.t. 23.0 ± 2.0 °C and 12 h light: dark cycle). The animals were fed with standard diet and water *ad libitum*.

3.4. Analgesic activity evaluation using acetic acid induced writhing assay

The method of Koster et al. (1959) was adopted with minor modification. The animals were orally fed with the extracts, vehicles (for control groups) at the specified doses (250 and 500 mg/kg body weight). Thirty minutes after administration of the extract and the vehicle, each animal was given 0.7% (v/v) solution of acetic acid (0.1 ml/10 g body weight) interperitoneally (i.p.) to induce abdominal contractions or writhing. Five minutes after the administration of acetic acid, the number of writhings for each animal was counted for 15 min. The number of writhings in the control was taken as 100% and percent inhibition was calculated as follows:

$$\% \text{ Inhibition of writhing} = 100 - (\text{treated mean/control mean}) \times 100$$

For comparison, the same experiment was carried out with a positive control group treated orally with aspirin (Square Pharmaceuticals Ltd., Bangladesh) at the dose of 150 mg/kg body weight.

3.5. Neuropharmacological studies

3.5.1. Pentobarbitone-induced sleeping time test

This method was carried out following the method described by Wambebe (1985). The animals were randomly divided into four groups consisting of five mice each. The test groups received the extract at specified doses (250 and 500 mg/kg body weight) while the positive control was treated with diazepam (1 mg/kg) and the control with vehicle (1% Tween 80 in water). All the administrations were carried out orally. After 30 min, pentobarbi-

Table 3: Effect of *P. foetidus* leaf extract in hole cross test*

Treatment	Dose (mg/kg, p.o.)	Number of hole crossed						
		0 min	30 min	60 min	90 min	120 min	180 min	240 min
Control (vehicle, 10 ml/kg)		8.4 ± 0.51	6.2 ± 0.37	5.6 ± 0.4	5.4 ± 0.51	5 ± 0.316	5.6 ± 0.67	5.4 ± 0.51
P. foetidus	250	8.0 ± 0.70	3.8 ± 0.73[c]	2.2 ± 0.66[b]	2.6 ± 0.40[b]	2.4 ± 0.24[a]	2.8 ± 0.37[b]	3.0 ± 0.44[b]
	500	8.8 ± 0.86	3.0 ± 0.70[b]	1.4 ± 0.51[a]	2.2 ± 0.37[a]	2.0 ± 0.31[a]	2.6 ± 0.24[b]	2.4 ± 0.51[b]

* Values are mean ± S.E.M. (n = 5)
[a] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.02$ vs control, Student's t-test

Table 4: Effect of *P. foetidus* leaf extract in open field test*

Treatment	Dose (mg/kg, p.o.)	Number of movements						
		0 min	30 min	60 min	90 min	120 min	180 min	240 min
Control (vehicle, 10 ml/kg)		114 ± 3.59	74 ± 5.33	58.4 ± 4.77	56.8 ± 2.083	53.6 ± 3.8	49.4 ± 1.63	47.2 ± 2.05
P. foetidus	250	119.8 ± 1.65	50 ± 3.53[b]	21.6 ± 2.65[a]	8.4 ± 0.51[a]	7.0 ± 0.70[a]	8.4 ± 1.63[a]	10 ± 1.02[a]
	500	118 ± 1.22	25.3 ± 1.63[a]	7.6 ± 0.51[a]	5.0 ± 1.14[a]	5.4 ± 0.51[a]	7.0 ± 1.0[a]	9.0 ± 0.51[a]

* Values are mean ± S.E.M. (n = 5)
[a] $P < 0.001$, [b] $P < 0.01$ vs control, Student's t-test

tone (50 mg/kg i.p.) was administered to each mouse to induce sleep. The animals were observed for the latent period (time between pentobarbitone administration to onset of sleep) and duration of sleep (time between the loss of righting reflex to the recovery of righting reflex).

3.5.2. Hole cross test

The method was adopted as described by Takagi et al. (1971). A steel partition was fixed in the middle of a cage having a size of $30 \times 20 \times 14$ cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The number of passages of a mouse through the hole from one chamber to other was counted for a period of 3 min on 0, 30, 60, 90, 120, 180 and 240 min during the study period.

3.5.3. Open field test

This experiment was carried out as described by Gupta et al. (1971). The animals were divided into control and test groups containing five mice in each. The test groups received *P. foetidus* leaf extract at the doses of 250 and 500 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water). The floor of an open field of half square meter was divided into a series of squares, each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min on 0, 30, 60, 90, 120, 180 and 240 min during the study period.

3.6. Statistical analysis

All data were presented as mean ± SEM. The level of significance was assessed by the student's t-test.

Acknowledgments: The work has been supported by a grant from Pharmacy Discipline, Khulna University, Bangladesh. The authors are grateful to Md. Abdul Matin and Md. Asraful Alam for their technical assistance.

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