J. Pharm. Pharmacol. 1999, 51: 313–318
Received October 6, 1998
Accepted October 7, 1998

Neuropsychopharmacological Profile of the Methanolic Fraction of *Bryophyllum pinnatum* Leaf Extract

SIDDHARTHA PAL, T. SEN AND A. K. NAG CHAUDHURI

Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Calcutta – 700 032, India

Abstract

Neuropharmacological studies were conducted in experimental animals (rats and mice) with the methanolic fraction of *Bryophyllum pinnatum* leaf extract.

The fraction produced alteration of behaviour pattern, caused dose-dependent potentiation of pentobarbitone sleeping time and had significant analgesic activity. Significant reduction of exploratory behaviour and loss of residual curiosity were among the effects observed with the fraction. The observations suggest that the methanolic fraction of *Bryophyllum pinnatum* possesses a potent CNS depressant action.

Bryophyllum pinnatum (Lam) Kurz (Cracculaceae) is a perennial herb growing widely throughout the hot and humid parts of India. In traditional medicine, the leaves of this herb have been advocated for use in the treatment of bruises, wounds, boils and insect bites (Kirtikar & Basu 1975). A literature survey revealed that the leaf extract of *B. pinnatum* has been reported to possess antifungal (Misra & Dixit 1979) and antibacterial (Mehta & Bhat 1952) actions. In the course of pharmacological studies, analgesic and anti-inflammatory activity (Pal & Nag Chaudhuri 1989), antiulcer activity (Pal & Nag Chaudhuri 1991) and probable mechanism of anti-inflammatory activity (Pal & Nag Chaudhuri 1992) of the methanolic fraction of the leaf extract have already been reported from this laboratory. Here, we report the neuropharmacological aspect of the methanolic fraction of the extract of *B. pinnatum* leaves in experimental animals.

Materials and Methods

Plant material

Authenticated leaves of *B. pinnatum* (supplied by United Chemicals and Allied Products, Calcutta, India) were extracted as described elsewhere (Pal & Nag Chaudhuri 1990).

Correspondence: A. K. N. Chaudhuri, Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Calcutta – 700 032, India.

Experimental animals

The experiments were conducted using Swiss A mice (18–22 g) and Charles-Foster rats (120–180 g) obtained from Ghosh & Co., Calcutta. The animals were used after an acclimatisation period of 10 days and were housed in standard metal cages and had free access to food and water.

Behavioural changes and toxicity studies

Groups of mice (n = 10), after intraperitoneal administration of different doses of methanolic *B. pinnatum* leaf extract (100, 200, 300 and 500 mg kg^{-1}), were observed at 30-min intervals for up to 2 h for probable behavioural changes (Irwin 1962).

For the toxicity study, groups of male mice (n = 10) were injected with different doses of the leaf extract and the mortality were recorded after 24 h.

Effect on pentobarbitone sleeping time

Groups of 10 male mice were injected with pentobarbitone sodium (40 mg kg^{-1}, i.p.) 15 min after intraperitoneal administration of either control vehicle (physiological saline) or *B. pinnatum* leaf extract (100, 200 and 400 mg kg^{-1}). The time interval between losing and regaining of righting reflex was measured as sleeping time (Dandiya & Collumbine 1959).

Analgesic activity

The analgesic activity of the *B. pinnatum* methanolic leaf extract was measured against chemical and mechanical noxious stimuli in mice.

Chemical method (acetic acid-induced abdominal constrictions). This was carried out in groups of mice (n = 10) by noting the writhing responses produced by intraperitoneal administration of 300 mg kg^{-1} of 3% aqueous acetic acid 15 min after intraperitoneal injection of either control vehicle or leaf extract (100, 200 and 300 mg kg^{-1}) (Turner 1965).

Mechanical method (tail clip method). The mice, which tried to remove a rubber-covered clip applied to the base of their tail within 15 s were used in this test. The test was performed in groups of mice (10 in each) at 15 and 30 min after administration of drugs (Bianchi & Franceschini 1954).

Anticonvulsant activity

Pentylenetetrazole-induced convulsion. Pentylenetetrazole (100 mg kg^{-1}, i.p.) was injected into the groups of mice (n = 10) pretreated 30 min earlier either with control vehicle or *B. pinnatum* methanolic leaf extract (100, 200, 300 and 500 mg kg^{-1}) and the mortality was recorded in each group (Soaje-Echaque & Lim 1962).

Antagonism to strychnine-induced convulsion. Control vehicle or *B. pinnatum* leaf extract (in different doses), were injected to groups of mice (n = 10), 30 min before administration of strychnine (3 mg kg^{-1}, i.p.). The number of deaths in each group were recorded after 4 h (Rudzik et al 1973).

Body temperature

Rectal temperature was recorded with an electronic telethermometer (Apex India, DCT - 1002) at predetermined times in groups of mice (n = 10) before and after the administration of either control vehicle or *B. pinnatum* leaf extract (100, 200, 300 mg kg^{-1}), for 4 h.

Effect on conditioned avoidance response

Male rats were trained to climb a pole on hearing the sound of a buzzer in order to avoid an electrical shock passed through the grid floor fifteen seconds later. On further training of these rats (trained for conditioned avoidance response (CAR)), the animals climbed the pole immediately on being placed in the pole-climbing apparatus. This phenomenon was termed as secondary conditioned response (SCR). The rats which showed a correct SCR in at least 10 consecutive trials were selected for further experimentation. Groups of selected animals were injected with either control vehicle or *B. pinnatum* leaf extract (100, 200 and 300 mg kg^{-1}) and were tested 30 min later and thereafter at the end of each hour for 3 h (Maffi 1959).

Effect on exploratory behaviour pattern

Head-dip test. Female mice (n = 10), 30 min after injection with control vehicle or *B. pinnatum* leaf extract (50, 100 and 200 mg kg^{-1}), were placed singly on a wooden board with 16 evenly spaced holes and the number of times the head was dipped into the holes during 3-min trials was counted. The same experiment was repeated after the administration of a mixture of amphetamine and leaf extract (1 : 10) (Dorr et al 1971).

Y-maze test. Female rats (n = 6), pretreated with either control vehicle or *B. pinnatum* leaf extract (100, 200 and 300 mg kgM^{-1}) 35 min before the experiment, were placed singly in a Y-shaped runway (33 cm × 38 cm × 13 cm) for 5 min and the number of times that the rats entered the arm of the maze with all four feet (classed as an entry) were counted (Rushton et al 1961).

Evasion test. Those mice which escaped within 5 min from a rectangular box with an inclined plane by which the mice could escape from the box were selected for further testing. Fifteen minutes after administration of control vehicle or *B. pinnatum* leaf extract (100, 200 and 300 mg kg^{-1}), the mice in each group (n = 10) were placed in the box again. The number of mice remaining in the box after 5 min in each group was noted (Turner 1965).

Muscle relaxant activity

Chimney test. In a pyrex glass tube (30 cm long and 28 mm diameter) marked at a point 20 cm from its base, a mouse was introduced at the end nearest the mark. When the animal reached the other end of the tube, the tube was moved to the vertical position and immediately the mouse tried to climb backwards. Only those mice which reached the mark within 30 s were selected for further testing (Boissier et al 1961). Screened mice were injected intraperitoneally with either control vehicle or *B. pinnatum* leaf extract (100, 200 and 300 mg kg^{-1}) and were tested after 15 min as described above.

Effect on aggressive behaviour

Fighting was produced in pairs of male albino mice confined in a 2-L inverted beaker (15 cm diameter and 20 cm high) by subjecting them to an electrical footshock (interrupted direct current of 3 mA, 400 V stimulus intensity of 0·2 s duration at a frequency of 5 shocks s^{-1}) using an apparatus consisting of a grid floor composed of parallel stainless steel rods. Only those mice which showed at least 1 fighting episode in 3 min were selected for further experimentation (Tedeschi et al 1959). Tests were performed on 8 pairs of previously screened mice after administration of either control vehicle or *B. pinnatum* leaf extract (100, 200, 300 and 500 mg kg^{-1}).

Effect on brain GABA content

The brain gamma aminobutyric acid (GABA) content in mice (n = 5) was estimated according to the method of Lowe et al (1958). Animals were killed by decapitation at predetermined time intervals after the intraperitoneal administration of vehicle or extract of *B. pinnatum leaf* (300 mg kg^{-1}). Brains were rapidly removed, blotted, weighed and placed in 5 mL of ice-cold trichloroacetic acid (10% w/v), then homogenized and centrifuged at 10 000 *g* for l0 min at 0°C. A sample (0·1 mL) of tissue extract was placed in 0·2 mL of 0·14 M ninhydrin solution in 0·5 M carbonate–bicarbonate buffer (pH 9·95), kept in a water bath at 60°C for 30 min, then cooled and treated with 5 mL of copper tartrate reagent (0·16% disodium carbonate and 0·03% copper sulphate and 0·0329% tartaric acid). After 10 min the fluorescence at 377/455 nm in a Shimadzu RP-5000 spectrofluorimeter (Japan) was recorded.

Statistical analysis

Statistical comparisons and significance levels were analysed with Student's *t*-test or Chi-square test (as applicable).

Results

Behavioural responses

The leaf extract of *B. pinnatum* in doses up to 2400 mg kg^{-1} did not cause any mortality in groups of male albino mice during the 24-h period after injection. However a significant change in behaviour of the mice treated with extract (100 mg kg^{-1} and above, i.p.) was observed. The animals became remarkably quiet and marked decrease of locomotor activity, lasting for about 2 h, was observed. However, there were no disturbances in maintenance of equilibrium and no ptosis even with much higher doses (500 mg kg^{-1}, i.p.).

Pentobarbitone-induced sleeping time

Pretreatment with *B. pinnatum* leaf extract significantly potentiated pentobarbitone-induced sleeping time in mice in a dose-dependent fashion (Table 1).

Analgesic activity

The *B. pinnatum* leaf extract (at doses of 100 mg kg^{-1} and above, i.p.) demonstrated significant analgesic activity against acetic acid-induced abdominal constriction (Table 2). However, in the tail-clip test no analgesic action could be detected (at doses of 300 mg kg^{-1}, i.p.).

Anticonvulsant action

Leaf extract up to a dose of 500 mg kg^{-1} (i.p.) could not be found to offer any significant protection either against pentylenetetrazole-induced convulsion or strychnine-induced convulsion and lethality, although in pentylenetetrazole-induced

Table 1. Effect of *B. pinnatum* leaf extract and chlorpromazine on pentobarbitone-induced sleeping time in mice.

Treatment	Dose (mg kg^{-1})	Mean sleeping time (min)
Control vehicle		38 ± 4
Leaf extract	100	54 ± 4*
Leaf extract	200	67 ± 4**
Leaf extract	400	100 ± 5**
Chlorpromazine	4	138 ± 6**

Results are expressed as means ± s.e.m. (n = 10). *$P < 0.05$, **$P < 0.001$ compared with control.

Table 2. Effect of *B. pinnatum* leaf extract on acetic acid-induced abdominal constriction in mice.

Treatment	Dose (mg kg^{-1}, i.p.)	Number of constrictions
Control	–	31·40 ± 1·16
Leaf extract	100 mg kg^{-1}	22·10 ± 1·10*
Leaf extract	200 mg kg^{-1}	13·30 ± 1·18*
Leaf extract	300 mg kg^{-1}	8·10 ± 0·98*
Aspirin	100 mg kg^{-1}	7·30 ± 0·69*

Results are expressed as means ± s.e.m. (n = 10). *$P < 0.001$ compared with control.

convulsion 500 mg kg^{-1} (i.p.) of the extract caused delay in time of death (14 ± 3 min) with respect to control vehicle (3 ± 1 min) treatment.

Body temperature
Administration of *B. pinnatum* leaf extract up to a dose of 300 mg kg^{-1} (i.p.) did produce an alteration of body temperature in mice.

Conditioned avoidance response
Leaf extract was not effective in blocking SCR and CAR of trained rats. Chlorpromazine (20 mg kg^{-1}, i.p.) was found to block both SCR and CAR in all the animals.

Exploratory behaviour pattern
In the head-dip test in mice, *B. pinnatum* leaf extract caused a significant reduction in head-dip responses as compared with control. In another set of experiments, simultaneous administration of a mixture of amphetamine and leaf extract (at ratio of 1 : 10 by weight) caused a marked increase in head-dips occurred as compared with either leaf extract or amphetamine treatment alone (Table 3).

In Y-maze tests there was a remarkable decrease in the exploratory behaviour of mice treated intraperitoneally with *B. pinnatum* leaf extract in doses of 100 mg kg^{-1} and above as compared with control (Table 4).

Leaf extract (100 mg kg^{-1} and above; i.p.) caused a significant inhibition of residual curiosity in mice as observed in evasion tests (Table 5).

Table 3. Effect of *B. pinnatum* leaf extract, diazepam, amphetamine and a mixture of amphetamine and leaf extract (1 : 10) on the head-dip test in mice.

Treatment	Dose (mg kg^{-1})	No. of head dips in 3 min
Control vehicle	–	26 ± 2
Leaf extract	50	15 ± 2**
Leaf extract	100	5·2 ± 0·5**
Leaf extract	200	2·6 ± 0·4**
Diazepam	10	3·5 ± 0·4**
Amphetamine	5	25 ± 2
Amphetamine +leaf extract	5 +50	39 ± 1*

Results are expressed as means ± s.e.m. (n = 10). *$P < 0·01$, **$P < 0·001$ compared with control.

Table 4. Effect of *B. pinnatum* leaf extract and diazepam on the Y-maze test in rats.

Treatment	Dose (mg kg^{-1}, i.p.)	Mean entry in 5 min
Control	0·1 mL (10 g)$^{-1}$	11·67 ± 0·67
Leaf extract	100	3·83 ± 0·60*
Leaf extract	200	3·33 ± 0·71*
Leaf extract	300	1·67 ± 0·33*
Diazepam	10	2·17 ± 0·48*

Results are presented as means ± s.e.m. (n = 6). *$P < 0·001$ compared with control.

Muscle relaxant activity
In the chimney test, there was a significant loss of coordination and muscle tone in mice treated intraperitoneally with *B. pinnatum* extract in a dose of 300 mg kg^{-1} (Table 6).

Aggressive behaviour
On footshock-induced fighting behaviour, no inhibition of response in mice treated with leaf extract (up to a dose of 500 mg kg^{-1}, i.p.) could be observed.

Estimation of brain GABA content
The results showed that *B. pinnatum* leaf extract, at an intraperitoneal dose of 300 mg kg^{-1}, caused a significant increase in brain GABA concentration in mice (Table 7).

Discussion

The results of the present study indicate that the methanol fraction of the extract of *B. pinnatum* leaf produced definite alterations in general behaviour pattern, significant reduction in spontaneous motility, potentiation of pentobarbitone-induced sleeping time (in a dose-dependent fashion) and significant analgesic action against acetic acid-induced abdominal constriction. All of these findings are suggestive of a potent CNS-depressant action of the extract.

Though the extract was found to possess significant analgesic action against acetic acid-induced abdominal constriction, no such activity could be found (up to a dose of 300 mg kg^{-1}, i.p.) in another model of analgesia (tail-clip test), thereby indicating that the leaf extract is effective against inflammatory pain but that its analgesic action is not mediated through the CNS.

The extract of *B. pinnatum* leaf failed to produce any alteration of body temperature in normothermic mice in the current investigation, although we had previously reported (Pal & Nag Chaudhuri 1989) that it possesses significant antipyretic properties. This is not surprising since it is well known that certain psychoactive CNS-depressant

Table 5. Effect of *B. pinnatum* leaf extract and diazepam on the evasion test in mice.

Test substance	Dose (mg kg^{-1}, i.p.)	No. of mice remaining in box after 5 min	No. of mice residual	% mice showing curiosity
Control vehicle	–	10	0	100
Leaf extract	100	10	6	40
Leaf extract	200	10	8	20
Leaf extract	300	10	9	10
Diazepam	10	10	10	0

Results are presented as means ± s.e.m. (n = 10).

Table 6. Effect of *B. pinnatum* leaf extract and diazepam on the chimney test in mice.

Test substance	Dose (mg kg^{-1}, i.p.)	No. of mice	No. of mice failed in the test	% Failure in the test
Control vehicle	0·1 mL (10 g)$^{-1}$	10	0	0
Leaf extract	100	10	0	0
Leaf extract	200	10	2	20
Leaf extract	300	10	5*	50
Diazepam	10	10	10**	100

n = 10. *$P < 0{\cdot}05$, **$P < 0{\cdot}00$ 1 compared with control (by chi square test).

Table 7. Effect of *B. pinnatum* leaf extract on brain GABA content in mice.

Treatment	Dose (mg kg^{-1}, i.p.)	GABA level (μg brain tissue)	% Increase
Vehicle	–	389·75 ± 7·6	–
Leaf extract	300	479·2 ± 8·1	18·6

Results are presented as means ± s.e.m. (n = 5). *$P < 0{\cdot}01$.

drugs (e.g. antipsychotics) reduce temperature both in normal and pyretic conditions (Baldessarini 1985; Bradley 1989) while other psychoactive CNS-depressant drugs (e.g. diazepam) are devoid of any such actions (Bartholini et al 1973). Accordingly, in view of the observed antipyretic effect of the extract, further investigations were performed using some other experimental models commonly applied for evaluation of psychopharmacological agents e.g. exploratory behaviour pattern, muscle relaxant activity and electroshock-induced fighting in mice.

The leaf extract produced a significant decrease in exploratory behaviour pattern as evident from the results of head-dip, Y-maze and evasion tests. Furthermore, in the head-dip test, concurrent administration of the extract in combination with amphetamine gave rise to a significant increase in head dips. Both reduction in head dip caused by extract treatment alone and increase in head dip on treatment of extract together with amphetamine are in conformity with the findings of Dorr et al (1971), with commonly used tranquillising drugs.

Conditioned avoidance response in rats has been used to evaluate the effect of CNS active drugs. It was found that many such drugs in suitable doses may suppress CAR/SCR or both, and such observations are useful in providing an experimental approach for studying the specific behavioural action of drugs. The *B. pinnatum* leaf extract neither anatagonized the conditioned avoidance response nor the secondary conditioned response. It might be mentioned in this context that hypnotics and centrally acting drugs (e.g. barbitone, pentobarbitone, mephensin etc.) are also incapable of producing any significant specific blockade of either SCR or CAR (Maffi 1959).

The leaf extract did not inhibit electroshock-induced fighting in mice, thereby demonstrating failure to suppress aggressive behaviour pattern. The extract also could not offer any protection against convulsion induced by either pentylenetetrazole or strychnine in mice (although our results indicate that the leaf extract prolonged the time of death after convulsion). Both of these observations suggest that the CNS-depressant action of the extract differs in all probability from the profile of anxiolytic drugs (Rang et al 1996).

The *B. pinnatum* leaf extract was found to produce significant inhibitory effect in the chimney test thereby demonstrating muscle relaxant activity (Rang et al 1996).

GABA is known as an inhibitory neurotransmitter in a number of CNS pathways. The widespread distribution of GABA, coupled with the fact that virtually all neurons are sensitive to its inhibitory effect, indicates that GABA function is ubiquitous in the brain. Studies have also shown that GABA serves as a transmitter at about 30% of all the synapses in the CNS (Rang et al 1996). Our studies with *B. pinnatum* indicate that the leaf extract significantly increased brain GABA content in mice. According to a study conducted by Saad (1972), CNS-depressant drugs increased brain GABA content in mice, and these findings are in agreement with our studies with *B. pinnatum* leaf extract.

However, the precise nature and mechanism of the CNS-depressant activity of the extract is difficult to predict at this moment and further investigations are necessary before any definite conclusion can be drawn in this respect. Further work in this regard is in progress in our laboratory.

Acknowledgement

Partial financial assistance to Siddhartha Pal from the University Grants Commission, New Delhi, India is gratefully acknowledged.

References

Baldessarini, R. J. (1985) Drugs and the treatment of psychiatric disorders. In: Gilman A. G., Goodman, L. S., Rall, T. W., Murad, F. (eds). The Pharmacological Basis of Therapeutics. 7th edn, Macmillan, New York, pp 387–445

Bartholini, G., Keller, H., Pieri, L., Pletscher, A. (1973) The effect of diazepam on the turnover of cerebral dopamine. In: Garattini, S., Mueseini, E., Randall, L. O. (eds) The Benzodiazepines. Raven Press, New York, pp 235–240

Bianchi, C., Franceschini, G. (1954) Comparative evaluation of antipyretic activity in normothermic and pyrexial mice. Br. J. Pharmacol. 9: 280–282

Boissier, J. R., Dremont, C., Robins, R., Pagny, J. (1961) Tentative de pharmacologie previsionelle dans de domain de neuroleptiquee: actions sedative centralle et adrenolytique de la N (dimethoxy-3,4 phenethyl) N′(chloro-2 phenyl) piperazine. Arch. Int. Pharmacodyn. 133: 29–32

Bradley, B. P. (1989) Neuroleptic drugs. In: Introduction to Neuropharmacology. Wright, London, pp 201–219

Dandiya, P. C., Collumbine, H. (1959) Studies on Accrue; culmus. Pharmacological actions of essential oils. J. Pharmacol. Exp. Ther. 125: 353–359

Dorr, M., Joycee, D., Porsolt, R. D., Steinberg, H., Summerfield, A., Tomkiewicz, M. (1971) Persistence of dose-related behaviour in mice. Nature 231: 121–123

Irwin, S. (1962) Drug screening and evaluative procedures. Science 136: 123–136

Kistikar, K. R., Baeu, B. D. (1975) Crassulaceae. In: Blaster, B., Caius, J. F., Mhaskar, K. S. (eds) Indian Medicinal Plants. Vol 11, Lalit Mohan Basu, India, pp 999–1000

Lowe, I. P., Robins, E., Eyermen, G. S. (1958) The fluorimetric measurement of glutamic decarboxylase and its distribution in brain. J. Neurochem. 3: 8–16

Maffi, G. (1959) The secondary conditioned response of rats and the effects of some psychopharmacological agents. J. Pharm. Pharmacol. 11: 129–139

Mehta, S., Bhat, J. V. (1952) Studies on Indian medicinal plants II: bryophyllin, a new antibacterial substance from the leaves of *Bryophyllum calyciunum* salsib. J. University of Bombay 21: 21–25

Misra, S., Dixit, S. N. (1979) Antifungal activity of leaf extract of some higher plants. Acta Botanica Indica 7: 147–150

Pal, S., Nag Chaudhuri, A. K. (1989) Preliminary studies on the anti-inflammatory and analgesic activities of *Bryophyllum pinnatum* (Lam). Med. Sci. Res. 17: 561–562

Pal, S., Nag Chaudhuri, A. K. (1990) Anti-inflammatory action of *Bryophyllum pinnatum* leaf extract. Fitoterapia 61: 527–533

Pal, S., Nag Chaudhuri, A. K. (1991) Studies on the anti-ulcer activity of a *Bryophyllum pinnatum* leaf extract in experimental animals. J. Ethnopharmacol. 33: 97–102

Pal, S., Nag Chaudhuri, A. K. (1992) Further studies on the anti-inflammatory profile of the methanolic fraction of the fresh leaf extract of *Bryophyllum pinnatum*. Fitoterapia 63: 451–459

Rang, H. P., Dale, M. M., Ritter, J. M. (1996) Chemical transmission and drug action in the central nervous system. In: Pharmacology. 3rd edn, Churchill Livingstone, Edinburgh, pp 491–561

Rudzik, A. D., Henter, J. B., Tang, A. H., Straw, R. N., Friis, W. (1973) Triazolobenzodiazepines, a new class of central nervous system-depressant compounds. In: Garattini, S., Mussini, S., Randall. L. O. (eds) The Benzodiazepines. Raven Press, New York, pp 285–297

Rushton, R., Steinberg, H., Tinson, C. (1961) Modification of the effects of an amphetamine barbiturate mixture by the past experience of rate (Y-shaped runway). Nature 192: 533–535

Saad, S. P. (1972) Administration of CNS depressant drugs like barbiturates, hydantoin and diazepam etc. can restore the isoniazid induced fall in brain GABA levels. J. Pharm. Pharmacol. 24: 839–840

Soaje-Echaque, E., Lim, R. K. S. (1962) Anticonvulsant activity of some carbonyl ureas. J. Pharmacol. Exp. Ther. 138: 224–228

Tedeschi, R. E., Tedechi, D. H., Mucha, A., Cook, L., Mattis,P. A., Fellows, J. E. (1959) J.Pharmacol. Exp. Ther. 125: 28–34

Turner, R. A. (1965) In: Screening Methods in Pharmacology, 113–117, Academic Press, N.Y. and London, pp 100–117, 87–89