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Pharmacology, Biochemistry and Behavior 79 (2004) 11–16

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Effect of leaves of *Butea frondosa* on stress, anxiety, and cognition in rats

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Received 24 August 2003; received in revised form 30 January 2004; accepted 17 May 2004
Available online 26 August 2004

Abstract

The plant *Butea frondosa* has been indicated in the Indian system of medicine as a plant augmenting memory and as a rejuvenator. The effect of oral administration of the aqueous and alcoholic extracts of the leaves was assessed on stress, cognitive function, and anxiety in albino rats. The antistress activity was evaluated using cold restraint induced ulcers and leukocyte count after subcutaneous injection of milk. The aqueous extract provides protection against stress-induced gastric lesions while both the alcoholic as well as the aqueous extract normalizes the white blood cell count. Effect on cognitive function was evaluated using Cook and Weidley's pole apparatus. The results indicate that the aqueous extract and the alcoholic extract when administered at a dose of 300 mg/kg for a period of 7 days augment both the acquisition as well as the retention of memory of learned task. The absence of an increase in the occupancy of the open arm in the elevated plus maze and in the number of head dips in the hole-board paradigms indicates that both the extracts are devoid of anxiolytic activity. Nootropic activity was compared using piracetam (100 mg/kg po) as the standard, while for anxiolytic and antistress activity, diazepam (1.0 mg/kg ip) was employed as the standard drug. It is concluded that the aqueous and alcoholic extract of *B. frondosa* possesses antistress and weak nootropic activity.

Keywords: *Butea frondosa*; Leaves; Nootropic activity; Antistress activity

1. Introduction

The Indian system of medicine is replete with medicinal plants claimed to promote learning, memory and intelligence (Satyavati, 1995). Plants like *Bacopa monniera* (Singh and Dhawan, 1992), *Azadirachta indica* (Jaiswal et al., 1994), *Withania somnifera* (Bhattacharya et al., 1995), as well as *Ocimum sanctum* (Rodrigues et al., 1999) have been investigated for their effect on cognitive functions of the brain. These plants have been grouped under the general class of rejuvenators, that is, drugs that counter the degenerative changes associated with ageing. Additionally, some of these plants act specifically in augmenting the cognitive functions of the brain and are akin to pharmacological agents termed as nootropic agents. These plants have also been investigated for their antistress activity (Bhargava and Singh, 1981; Dadkar et al., 1988; Satyavati, 1995) as they have been proved to produce an increase in the nonspecific resistance of the organism (Brekhman and Dardymov, 1969), thereby helping the organism to cope with stressful situations. The role of stress has been emphasized in diseases ranging from psychiatric and endocrine disorders to cognitive dysfunctions (Bhattacharya et al., 2000).

The plant *Butea frondosa* (*B. frondosa*; Family: Leguminosae) grows throughout India (Sivarajan and Balachandran, 1994) and has been extensively studied for its anthelmintic (Kaluysaraj and Kurup, 1962), antifertility (Lumas and Uniyal, 1966) and hepatoprotective activities (Wagner et al., 1986). The traditional system of medicine claims that the plant is a rejuvenator (Nadkarni, 1976). The flowers of the plant *B. frondosa* are reported to possess antistress (Bhatwadekar et al., 1999) and nootropic (Gawale et al., 2001) activities, but to date, no such study has been conducted on the leaves of the plant. The present study investigates the nootropic, anxiolytic and antistress activities of the aqueous and the alcoholic extracts of the leaves of the plant *B. frondosa*.

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doi:10.1016/j.pbb.2004.05.022

2. Materials and methods

2.1. Plant material

The plant was collected in the month of September from Kasara village, Maharashtra, India and was authenticated at the Botanical Survey of India, Pune (Voucher specimen No. 165413).

2.2. Preparation of extracts

The shade-dried leaves of the plant were powdered and subjected to extraction. The aqueous extract was prepared by decoction (yield 24.36% w/w) and the alcoholic extract (yield 21.20% w/w) by Soxhlet's extractor using 50% ethanol. The extracts were concentrated under reduced pressure and used for the neuropharmacological investigation.

2.3. Animals

Wistar Albino rats of both sex weighing 180–200 g were used for the study. The animals were housed in groups of six, under standard laboratory conditions of temperature (25 ± 2 °C), lighting (0800–2000 h), and relative humidity (50 ± 5%), with food (pellets by Hindustan Lever, Mumbai, India) and water freely available. Animals were fasted overnight prior to drug administration. All experiments were carried out during the light period. (0800–1600 h). The Institutional Animal Ethical Committee approved the protocol of the study.

2.4. Drugs

Piracetam, (Cerecetam, 400 mg/tablet, Intas Laboratories, India) and diazepam (Calmpose, 5 mg/tablet, Ranbaxy Laboratories, India) were used in this study. They were prepared for administration in the same manner as that for the plant extracts as explained below. The solvents used were of analytical grade.

2.5. Administration of the extracts

Suspensions of the aqueous and alcoholic extracts were prepared in distilled water using Tween 80 (0.2% v/v) as the suspending agent. The extracts were administered in a dose of 150 and 300 mg/kg to rats by oral route, 60 min before the test procedures. Control groups were given only the vehicle (0.2% v/v Tween 80 solution) in volume equivalent to that of the plant extracts and drugs.

2.6. Acute toxicity and effect on gross behavior

The acute toxicity test was carried out according to the OECD guidelines. The aqueous and alcoholic extracts were administered orally in doses of 100, 200, 400 and 800 mg/kg to groups of rats ($n = 6$) and the percent mortality was noted after 24 h. The effect on gross behavior was observed (Turner, 1965). The procedure involved an initial phase of undisturbed observations, for a period of 15 min followed by a manipulative phase during which the animals were subjected to the least provoking stimuli. In the initial phase, the animals were observed for body positions, locomotion, rearing, tremors, gait, and in the later phase, the effect on passivity, grip strength, pain response, stereotypy, vocalization and righting reflex was noted.

2.7. Effect on motor coordination

The effect on motor coordination was assessed using a rotarod apparatus (Dunham and Miya, 1957). All the animals were trained to remain for at least 4 min on the rod rotating at a speed of 10 rpm. Only those animals performing up to the required parameter were included in the test. Both the aqueous and alcoholic extracts were administered at 150 and 300 mg/kg po to separate groups of animals. The animals were subsequently tested for their performance on the rotarod 60 min after the administration of the plant extracts. The reference group received diazepam in a dose of 1.0 mg/kg ip 30 min before the test.

2.8. Assessment of antistress activity

2.8.1. Cold restraint stress (CRS)-induced ulcer

Overnight fasted rats were divided into groups of six each and they received the extracts, the vehicle or the reference drug diazepam (1.0 mg/kg ip) 1 h prior to being subjected to CRS. Rats were immobilized by placing them in plastic restraint boxes which were placed in a refrigerator at a temperature of 4–7 °C for 2 h. The rats were killed by cervical dislocation. The stomach was cut open from the greater curvature and the inner surface of the stomach was examined for lesions. The ulcer index was computed using the following formula: ulcer index = $10/X$, where X = total mucosal area/total ulcerated area (Senay and Levine, 1967).

2.8.2. Milk-induced leucocytosis

Subcutaneous injection of milk is known to produce an infection-like condition by acting as an antigen and increasing the white blood cell count. Antistress activity can be evaluated on the basis of the capacity of the drug to prevent this stress-induced increase in white blood cell count. (Brekhman and Dardymov, 1969; Bhargava and Singh, 1981). Animals were divided into groups of six each, and they received either the extracts, the vehicle or the reference drug diazepam (1.0 mg/kg ip) 1 h prior to the injection of milk 4 ml/kg sc. Twenty-four hours later, blood was withdrawn from the retro orbital plexus of the animals and the white blood cell count was computed using Neubauers chamber. (Brekhman and Dardymov, 1969).

2.9. Assessment of nootropic activity

The nootropic activity was assessed using the active avoidance paradigm (Cook and Weidley, 1957). The apparatus consisted of a soundproof experimental chamber with a grid floor which could be electrified and with a provision for a buzzer tone. The enclosure had a clear Perspex front sliding door, through which the animal could be introduced into the chamber. A wooden pole, screwed onto the inner surface of the lid of the chamber acted as the shock-free zone. In the assessment of nootropic activity, the stimulus provided was a foot shock of 6 mA given for a period of 10 s from the electrified grid floor. Rats were initially trained to escape the foot shock by climbing on to the pole, i.e., the shock free zone. This initial trial was carried out by having three trial sessions interspersed with an interval of 10 s. During each of the initial trials, the rats were allowed to explore the apparatus for 10 s. This was followed by the foot shock for 10 s. Only those rats which were sensitive to the foot shock and could climb the pole were included in the study. The animals were divided into six groups, each group containing six animals. The aqueous and alcoholic extracts (150 and 300 mg/kg po) were administered for a period of 7 days following which the training trial (TT) was conducted. This consisted of 10 trial sessions interspersed with an interval of 30 s. During each trial, the rats were allowed to explore the apparatus for 10 s, followed by a buzzer tone of 50 Hz (conditioned stimulus) for 10 s. This was followed by the foot shock for 10 s. The animal learned to associate the buzzer tone with the impeding foot shock and was capable of avoiding the foot shock on hearing the buzzer warning. Jumping onto the wooden pole, before the shock period, constituted an avoidance response (AR). The percent AR for the 10 trials was computed. Twenty-four hours later, a relearning trial (RT) composed of 10 trials was carried out and the number of ARs in the 10 trial sessions was noted. Piracetam 100 mg/kg po was used as the standard reference drug for comparison.

2.10. Assessment of anxiolytic activity

2.10.1. Elevated plus maze test

Anxiolytic activity was evaluated using the elevated plus maze (Pellow et al., 1985). The elevated plus maze consisted of two open arms (50 × 10 cm) crossed with two closed arms (50 × 10 × 40 cm). The arms were connected together with a central square (10 × 10 cm). The apparatus was elevated to the height of 70 cm in a dimly illuminated room. The rats were divided into groups of six and received the extracts, the vehicle or the standard reference drug diazepam (1.0 mg/kg ip). One hour postadministration, each rat was placed individually at the center of the elevated maze. The number of entries in the open and closed arm of the elevated maze during a period of 5 min and the duration of the stay in open arm were noted.

2.10.2. Hole-board apparatus

The hole-board apparatus consisted of a wooden chamber (40 × 40 × 25 cm) with 16 holes (diameter 3 cm) on the floor, elevated from the ground so that the rats could peep through the holes. The animals were divided into six groups of six animals each, and received either the extracts (150 and 300 mg/kg po), the vehicle or the standard reference drug diazepam (1.0 mg/kg ip). One hour following the treatment, each rat was placed individually in the apparatus and the number of head dips through the holes and the total time spent with the head dipped were noted during a 5-min test session. Increased exploratory behaviour, characterized by an increase in the number as well as duration of head dips is an indication of anxiolytic activity (File and Wardil, 1975).

2.11. Statistical analysis

The results are given as mean ± S.E.M. The data obtained was analysed by one-way analysis of variance (ANOVA) followed by Dunnett's test. Differences were considered significant at the 5% level.

3. Results

3.1. Acute toxicity

The rats treated with the aqueous and alcoholic extracts, 100–800 mg/kg po, exhibited normal behaviour. They were alert, with normal grooming, touch response and pain response. There were no signs of passivity, stereotypy or vocalization. Their motor activity and secretory signs were also normal. Alertness, limb tone and grip strength were normal and the animals did not show staggering gait or contractions. Both the extracts were found to be safe up to 800 mg/kg in rats.

3.2. Effect on motor coordination

Rats treated with 150 and 300 mg/kg po of the aqueous and alcoholic extracts were able to maintain equilibrium on the rotating rod for the complete observation period of 4

Table 1
Effect of extracts of *B. frondosa* and diazepam on ulcer index in cold-restraint stressed rats ($n = 6$)

Treatment (dose: mg/kg)	Ulcer index (mean ± S.E.M.)
Vehicle	1.133 ± 0.113
Diazepam (1.0)	0.156 ± 0.01 *
AqE (150)	0.586 ± 0.018 *
AqE (300)	0.273 ± 0.045 *
AlE (150)	0.426 ± 0.062 *
AlE (300)	0.421 ± 0.048 *

AqE—aqueous extract. AlE—alcoholic extract.
* $P < .05$, ANOVA followed by Dunnett's test.

Table 2
Effect of extracts of *B. frondosa* and diazepam on milk-induced leucocytosis in rats ($n=6$)

Treatment (dose: mg/kg)	WBC count before milk (mean ± S.E.M.)	WBC count after milk (mean ± S.E.M.)
Vehicle	8816.7 ± 602.7	15,708.3 ± 840.8
Diazepam (1.0)	8641.5 ± 774	10,825.0 ± 648.0 *
AqE (150)	6741.5 ± 775.5	9491.5 ± 895.5 *
AqE (300)	7358.0 ± 870.5	8583.0 ± 936.0 *
AlE (150)	7815.0 ± 581.0	11,065.0 ± 866.5 *
AlE (300)	7558.0 ± 826.0	8975.0 ± 803.5 *

AqE—aqueous extract. AlE—alcoholic extract.
* $P<.05$, ANOVA followed by Dunnett's test.

min. The animals did not exhibit any signs of motor incoordination.

3.3. Test for antistress activity

3.3.1. CRS-induced ulcers

Vehicle-treated rats subjected to cold restrained stress revealed gastric ulcers. Pretreatment with diazepam (1.0 mg/kg ip) as well as both the doses of the aqueous and alcoholic extract significantly reduced the ulcer index $F(5,30)=27.46$, $P<.05$. The observations are given in Table 1.

3.3.2. Milk-induced leucocytosis

Subcutaneous injection of milk in a dose of 4 ml/kg produced a significant increase in the leukocyte count 24 h after administration. The aqueous and alcoholic extract at both the dose levels and diazepam (1.0 mg/kg ip) significantly inhibited this increase in leucocyte count, $F(5,30)=148.96$, $P<.05$. The results are summarized in Table 2.

3.4. Test for nootropic activity

The Cook and Weidley's pole apparatus uses the percentage AR as an index for studying the nootropic activity.

Table 3
Effect of extracts of *B. frondosa* and piracetam on nootropic activity in rats when administered for 7 days using Cook and Weidley's pole apparatus ($n=6$)

Treatment (dose: mg/kg)	% Avoidance responses in TT (mean ± S.E.M.)	% Avoidance responses in RT (mean ± S.E.M.)
Vehicle	31.6 ± 4.4	41.6 ± 1.2
Piracetam (100)	68.3 ± 0.5 *	86.6 ± 0.4 *
AqE (150)	38.3 ± 7.2	53.3 ± 6.5
AqE (300)	55.0 ± 5.1 *	83.3 ± 3.8 *
AlE (150)	36.6 ± 7.3	46.6 ± 9.6
AlE (300)	56.6 ± 5.0 *	73.3 ± 7.7 *

AqE—aqueous extract. AlE—alcoholic extract.
TT—training trial.
RT—relearning trial.
* $P<.05$, ANOVA followed by Dunnett's test.

Table 4
Effect of extracts of *B. frondosa* and diazepam on exploratory activity of the rats using elevated plus maze apparatus ($n=6$)

Treatment (dose: mg/kg)	Time spent (s) (mean ± S.E.M.)		No. of entries (mean ± S.E.M.)	
	Open arm	Closed arm	Open arm	Closed arm
Vehicle	81.2 ± 26.5	215.5 ± 23.0	5.0 ± 0.7	17.5 ± 0.7
Diazepam (1.0)	313.5 ± 23.3 *	72.1 ± 14.6 *	25.0 ± 0.9 *	7.5 ± 0.9 *
AqE (150)	133.3 ± 18.2	164.0 ± 23.3	5.0 ± 1.5	17.5 ± 0.9
AqE (300)	147.0 ± 14.5	151.5 ± 12.9	7.5 ± 1.6	15.0 ± 1.5
AlE (150)	110.3 ± 15.0	190.5 ± 12.2	5.0 ± 1.2	20.0 ± 1.6
AlE (300)	148.3 ± 41.0	153.8 ± 18.9	7.5 ± 1.1	15.0 ± 0.8

AqE—aqueous extract. AlE—alcoholic extract.
* $P<.001$, ANOVA followed by Dunnett's test.

Piracetam (100 mg/kg po), the aqueous extract and the alcoholic extract (300 mg/kg po) administered for 7 days showed a statistically significant increase in the percentage AR in the TTs as well as in the RTs, $F(5,30)=5.71$, $P<.05$. The results are given in Table 3.

3.5. Test for anxiolytic activity

3.5.1. Elevated plus maze test

The aqueous as well as the alcoholic extracts at both the dose levels (150 and 300 mg/kg po) did not produce any significant increase in the number of entries nor in the duration of stay in the open arm, when compared to the vehicle control group. The reference drug diazepam (1.0 mg/kg ip) significantly increased ($P<.001$) the number of entries as well as the duration of stay in the open arms, indicating anxiolytic activity. The results are given in Table 4.

3.5.2. Hole-board apparatus

The groups treated with aqueous and alcoholic extracts did not show any significant increase in the number of dips or the duration of dips, when compared to vehicle-treated group. Only diazepam (1.0 mg/kg ip)-treated groups showed significant increases ($P<.001$) in the exploratory activity, thus indicating anxiolytic activity. The results are given in Table 5.

Table 5
Effect of extracts of *B. frondosa* and diazepam on exploratory activity in rats using hole-board apparatus ($n=6$)

Treatment (dose: mg/kg)	Duration of dip (s) (mean ± S.E.M.)	No. of dips (mean ± S.E.M.)
Vehicle	71.8 ± 11.6	15 ± 2.0
Diazepam (1.0)	151.3 ± 7.7 *	45 ± 3.2 *
AqE (150)	74.8 ± 11.8	18 ± 3.0
AqE (300)	81.0 ± 6.5	19 ± 1.1
AlE (150)	97.5 ± 16.6	17 ± 2.4
AlE (300)	93.5 ± 10.4	18 ± 1.1

AqE—aqueous extract. AlE—alcoholic extract.
* $P<.001$, ANOVA followed by Dunnett's test.

4. Discussion

The findings of the present study clearly indicate that the aqueous and alcoholic extracts at a dose of 300 mg/kg significantly improve the acquisition and retention of memory of the learned task as was seen in the increase in the percent ARs, thus demonstrating nootropic activity. The nootropics represent a class of agents that facilitate the integrative functions of the CNS, particularly the intellectual performance, learning capacity and memory. In our studies, the extracts demonstrated facilitatory effect on learning and memory only after treatment for a period of 7 days. This probably may be attributed to the involvement of neurotransmitters since the building of memory is augmented only when the levels of neurotransmitters are attenuated on repeated administration of the extracts. There is ample evidence that the central cholinergic system has a vital role in these processes (Hollander et al., 1986). Nootropics have also been demonstrated to interfere with serotonergic transmission and also have an inhibitory effect on noradrenaline function (Ogren, 1982; Nalini et al., 1995). However, controversial reports exist with respect to the involvement of dopamine in learning and memory processes (Nyback et al., 1979; Bhattacharya et al., 1989).

In our studies, both the aqueous and alcoholic extracts did not produce any significant change in the exploratory activity of the rats in the elevated plus maze and hole-board apparatus. This indicates a lack of anxiolytic activity. Generally, most of the anxiolytic agents have an adverse effect on memory and this has been reported in the case of benzodiazepines, which are commonly used as anxiolytics (Mrugnandam et al., 2000). An important point to be noted is that, recently, the plus maze model is also being used to study learning and memory processes in rodents. The impairment of learning and memory induced by scopolamine, an anticholinergic agent, was reflected by prolonged transfer latency from the open arm to the closed arm (Iyer et al., 1998). With respect to our findings, in contrast to that of diazepam, the extracts did not cause an increase in the number of entries into the open arm. It could thus be inferred that the rats retain the memory of the aversive quality of the open arm and this could probably be considered a significant finding with respect to the plant extracts. Moreover, it is also an advantage that the plant extracts did not demonstrate any effect on the muscle coordination, as evaluated by the rotarod model.

Our findings have also indicated that both the aqueous as well as the alcoholic extracts, at both the dose levels, provide a significant protection against the formation of gastric lesions induced by CRS. Furthermore, the white blood cell count in the milk-induced leucocytosis challenge model was normalized. Thus, these findings point out to the antistress activity of the plant extracts. There are studies that indicate that "stress" is one of the factors leading to cognitive deficits, anxiety and peptic ulcers (Mukherjee and Roy, 1990; Bhattacharya et al., 2000) Prolonged stress immobilization, extreme heat, cold and other stressors are associated with neuron cell degeneration in the hippocampal and other areas of the brain (Shukla et al., 2000). Additionally, stress is known to interfere with cognitive functions tending to retard the memory engram rather than the acquisition of learning (Kumar et al., 2000). Earlier studies conducted by various workers have revealed that several medicinal plants possess nootropic activity (Chintawar et al., 2002; Singh and Dhawan, 1997; Iyer et al., 1998), but only a few plants like *W. somnifera* possess both antistress (Singh et al., 1982) as well as nootropic activity (Bhattacharya et al., 1995). Similarly, our studies demonstrate that the plant *B. frondosa* possesses a combination of activities. As discussed earlier, these properties are complementary to each other and hence the plant could be a valuable contribution to the existing armamentarium of nootropic agent having antistress activity.

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