ELSEVIER

Pharmacology, Biochemistry and Behavior 69 (2001) 439–444

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Nootropic and anxiolytic activity of saponins of *Albizzia lebbeck* leaves

H.D. Une, V.P. Sarveiya[1], S.C. Pal, V.S. Kasture, S.B. Kasture*

Department of Pharmacology, MVP Samaj's College of Pharmacy, Nashik 422 002, Maharashtra State, India

Received 17 October 2000; received in revised form 27 February 2001; accepted 7 March 2001

Abstract

The effect of saponin containing, *n*-butanolic fraction (BF), extracted from dried leaves of *Albizzia lebbeck*, was studied on cognitive behavior and anxiety in albino mice. The elevated plus maze was used for assessment of both nootropic and anxiolytic activity. The nootropic activity was evaluated by recording the effect of BF (0, 10, 25, and 50 mg/kg) on the transfer latency, whereas anxiolytic activity was assessed by studying its effect on the duration of occupancy in the closed arm. Results showed significant improvement in the retention ability of the normal and amnesic mice as compared to their respective controls. Animals treated with BF (25 mg/kg) spent more time in the open arm in a dose-dependent manner. The BF was without any significant effect on motor coordination. However, it significantly inhibited passivity and hypothermia induced by baclofen (10 mg/kg), a $GABA_B$ agonist. The data emanated in the present study suggests involvement of gamma-aminobutyric acid (GABA) in the nootropic and anxiolytic activity of saponins obtained from *A. lebbeck*.

Keywords: Nootropic; Anxiolytic; Gamma-aminobutyric acid; Saponins; *Albizzia lebbeck*

1. Introduction

In recent years, there has been a phenomenal rise in the interest of scientific community to explore the pharmacological actions of herbs or to confirm the claims made about them in the official books of Ayurveda. Several plants have been reported to possess nootropic activity (Nadkarni, 1996). Drugs having different chemical structures exhibit nootropic activity. The alkaloids from *Vinca minor* and *Secale cornutum* (Gabryel and Trzeciak, 1994) and saponins, bacoside A and B from *Bacopa monnieri* (Singh and Dhavan, 1997) and ginsenoside Rb1 and Rb1-influene, the saponins from *Panax ginseng* (Ying et al., 1994), and total saponins isolated from red ginseng (Lee et al., 2000) are the active pharmacognostics responsible for enhancing cognitive behavior. Since the leaves of *Albizzia lebbeck* Benth (Fam. Mimosaceae) are rich in saponins (Pal et al., 1995), we investigated its nootropic activity.

The neurological basis of learning and memory remains controversial, despite extensive experimental and clinical studies. Although the role of the central cholinergic system is fairly well established, the role of other neurotransmitter system cannot be ignored. Increase in serotonergic neurotransmission can interfere with learning acquisition and memory consolidation (Jaffard et al., 1989). Piracetam, the classical nootropic agent, has been reported to augment rat brain dopaminergic activity (Nyback et al., 1979). Yamada et al. (1995) have reported the role of nitric oxide in learning and memory. These authors have reported decrease in the ratio of 5-hydroxy-indole acetic acid/5-hydroxytryptamine in the hippocampus and cortex and increase in the striatal 3,4-dihydroxyphenylacetic acid content. Kulkarni and George (1999) have reviewed the probable role of *N*-methyl-D-aspartate receptors in learning and memory. Lebrun et al. (2000) have demonstrated that a novel cognitive enhancer (*S*)-2,3-dihydro-[3,4] cyclopentano-1,2,4-benzothiadiazepine-1,1-dioxyde (S18986-1) facilitates postsynaptic responses modulating alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor mediated synaptic responses and promotes long-term potentiation and potentiates AMPA-induced release of noradrenaline in rat brain slices. In a randomized clinical trial, Missonnier et al. (1999) have shown involvement of noradrenergic system in the modulation of automatic attentional processing. Ogasawara et al. (1999) have reported involvement of cholinergic

* Corresponding author. Tel.: +91-253-346266.

E-mail address: kasture_sb@hotmail.com (S.B. Kasture).

[1] Present address: Faculty of Pharmacy, University of Manitoba, Winnipeg, MB, Canada.

0091-3057/01/$ – see front matter
PII: S0091-3057(01)00516-0

and gamma-aminobutyric acid (GABA) systems in the reversal of memory disruption by (+)-5-oxo-D-prolinepiperdinamide monohydrate (NS-105), a cognition enhancer. The compound inhibited memory impairment caused by scopolamine, baclofen ($GABA_B$ agonist), cerebral ischemia, and maximum electroshock.

For many years, it was thought that anxiolytic agents impair learning and memory, both in animals and humans. This assumption might be because of the involvement of amygdala in both memory and anxiety (Cahill and McGaugh, 1990; Davis, 1992) and the previous reports showing that anxiolytics particularly, the benzodiazepines (BZDs) impair (Arolfo and Brioni, 1991; Cole and Jones, 1995), whereas anxiogenic compounds like amphetamine and β-carboline improve memory (Janak and Martinez, 1992; Venault et al., 1986). The search for alternative to the BZD led to the finding that antagonists at the serotonin 5-HT_3 receptor cause anxiolysis and also improve memory simultaneously (Barness et al., 1990; Blackburn et al., 1993; Costall and Naylor, 1992). Several studies have shown that 5-HT_3 receptor antagonists have BZD like anxiolytic effects, but unlike the BZDs, these do not produce amnesia or may even facilitate learning (Arnsten et al., 1997; Chugh et al., 1991; De Souza Silva et al., 1993; Staubli and Xu, 1995). Recently, Hasenohrl et al. (1998) have shown dissociation between anxiolytic and hypomnestic effects for combined extracts of *Zingiber officinale* and *Ginkgo biloba*, as opposed to diazepam.

Many researchers have shown that some nootropic agents improve retention but not acquisition (Chugh et al., 1991; Hasenohrl et al., 1998; Jaiswal and Bhattacharya, 1992). The previous studies on the saponin containing fraction of *A. lebbeck* showed no significant effect on active avoidance learning in mice but improved object recognition. The brain concentration of GABA and dopamine was decreased whereas the serotonin level was increased (in press). In the present study, we studied the effect of *n*-butanolic fraction (BF) on retention of learned task and the anxiolytic activity of saponin containing the BF of *A. lebbeck*. In view of the link between the cholinergic and GABAergic transmission and reversal of baclofen amnesia by nootropic agent (Ogasawara et al., 1999), we also studied the effect of BF on baclofen-induced passivity, the behavior mediated via $GABA_B$ receptors (Olpe et al., 1993; Staubli and Xu, 1995).

2. Materials and methods

2.1. Isolation of saponins

Leaves of *A. lebbeck* were collected from the college campus and Dr. SC Pal of the Pharmacognosy department of the college confirmed its botanical identification. The specimen was deposited at the Botanical Survey of India, Pune (voucher specimen no. 166382). Saponins were isolated from dried leaves of *A. lebbeck* as described by Pal et al. (1995). In brief, shade dried leaves of *A. lebbeck* were defatted with petroleum ether (60–80°C) in Soxhlet's extractor. The marc was dried and extracted with methanol. The methanolic extract was evaporated to dryness in vacuum. The residue was suspended in water, extracted with ethyl acetate and *n*-butanol (3×300 ml each) and the solution was evaporated to dryness in vacuum to provide ethyl acetate (3.5 g), *n*-butanol (11.0 g), and water soluble (3.5 g) portions. The *n*-butanol soluble fraction (BF) was tested for the presence of saponins using haemolysis test and foam test as described earlier by Evans (1996).

2.2. Animals

Albino mice (Swiss, 20–22 g) and rats (Wistar, 125–150 g) were used in this study. The animals were allowed to acclimatize to the laboratory conditions for 10 days after their arrival. The animals were housed into groups of six under standard housing conditions. Animals were fasted overnight prior to drug administration and during the experiment. All experiments were carried out during the light period (08:00–16:00 h). Separate groups of subjects were used for various tests.

2.3. Drugs

Piracetam (Uni-UCB, India), scopolamine (German Remedies, India), (±)baclofen (Hindustan Ciba-Geigy, India) and ANXOL inj. (diazepam, Sigma, India) were used in this study. All drugs were dissolved in distilled water and administered intraperitoneally except stated otherwise.

2.4. Acute toxicity test

The BF was administered orally in doses of 10, 25, 50, 75, and 100 mg/kg body weight to groups of mice ($n = 10$), and percentage of mortality was noted after 24 h.

Table 1
Effect of BF of *A. lebbeck* on transfer latency

Treatment (mg/kg)	Transfer latency (s)		Inflexion ratio
	Day 1	Day 2	
Vehicle	44.5 ± 4.5	14.5 ± 3.45***	2.07 ± 0.8
Piracetam (100)	56.4 ± 10.5	10.6 ± 2.3***	4.32 ± 0.9*
BF (10)	49.6 ± 6.8	9.5 ± 1.6***	4.22 ± 0.8*
BF (25)	66.5 ± 10.2	11.3 ± 1.7***	4.9 ± 0.5**
BF (50)	78.4 ± 16.6	24.9 ± 4.8**	2.1 ± 0.6
Scopol (0.3)	33.2 ± 3.4	43.5 ± 4.32*	− 0.23 ± 0.16**
Piracetam (100) + Scopol (0.3)	60.3 ± 8.3	14.3 ± 1.5***	3.21 ± 0.2***
BF (25) + Scopol (0.3)	86.5 ± 12.4	22.7 ± 4.56***	2.81 ± 0.2***

$n = 6$; values are means ± S.E.M.; Scopol = Scopolamine.
* $P < .05$ vs. respective control (Student's *t* test).
** $P < .01$ vs. respective control (Student's *t* test).
*** $P < .005$ vs. respective control (Student's *t* test).

Table 2
Effect of BF of *A. lebbeck* on time spent in both the arms and number of entries in the arms

	Time spent (s)		No. of entries	
Treatment (mg/kg)	Open arm	Closed arm	Open arm	Closed arm
Vehicle	45.0 ± 3.2	210.6 ± 10.9	2.2 ± 0.9	4.5 ± 2.4
Diazepam (1.0)	147.0 ± 12.2**	102.5 ± 10.8**	12.4 ± 2.0**	8.1 ± 1.4
BF (10)	40.5 ± 4.7	208.6 ± 14.6	6.1 ± 0.9*	4.2 ± 1.1
BF (25)	120.5 ± 7.4**	124.4 ± 8.5**	8.1 ± 0.9**	6.2 ± 2.1
BF (50)	115.4 ± 9.7**	153.4 ± 10.0**	4.3 ± 0.5*	5.7 ± 0.9

$n = 6$.
* $P < .01$ vs. vehicle (Student's t test).
** $P < .001$ vs. vehicle (Student's t test).

2.5. Test for neurotoxicity

In this test, a knurled rod (2.54 cm in diameter) was rotated at a speed of 10 rpm. All animals were trained to remain on the rotating rod for 5 min. A normal mouse could maintain its equilibrium for long periods. In a drug-treated mouse, the neurological deficit was indicated by inability of the mouse to maintain equilibrium for 3 min in each of three trials as described earlier (Dunham and Miya, 1957). BF was administered in doses of 10, 25, or 50 mg/kg, and the animals were tested for neurological deficit 30 min after the drug. The reference group received diazepam in a dose of 1.0 mg/kg ip.

2.6. Assessment of nootropic activity

An elevated plus maze consisting of two open arms (35×6 cm) and two enclosed arms ($35 \times 6 \times 15$ cm) was used. The maze was elevated to the height of 25 cm. Mice were placed individually at the end of an open arm facing away from the central platform and the time it took to move from the end of open arm to either of the closed arms (transfer latency, TL) was recorded (Ogasawara et al., 1999). On the first day, mice were allowed to explore the plus maze for 3 min after the measurement of TL. On the following day, male mice received vehicle, piracetam (100 mg/kg) or BF (10–50 mg/kg) 30 min before their placement on the elevated plus maze as before and TL was noted again. The TL was expressed as retention after 24 h by calculating the "inflexion ratio (IR)" using the formula described earlier by Jaiswal and Bhattacharya (1992):

$$\mathrm{IR} = (L_1 - L_0)/L_0$$

where L_0 = transfer latency after 24 h and L_1 = initial transfer latency in seconds.

2.7. Anxiolytic activity

Male mice were individually placed in the centre of the plus maze (having dimension same as used for measurement for TL) facing an enclosed arm. The time spent by the mouse during the next 5 min on the open and closed arms was recorded along with the number of entries into the open and closed arms (Pellow and File, 1986). Animals were treated with vehicle, BF (10–50 mg/kg), or diazepam (1 mg/kg) 30 min before their placement on the maze. Each group contained six animals.

2.8. Behavioral studies

2.8.1. Baclofen-induced hypothermia

Baclofen-induced hypothermia was used to assess the effect of drugs influencing GABA mediated behavior (Jackson and Nutt, 1991). Albino rats were taken into groups of five each. BF (25 mg/kg) was administered 30 min before baclofen and rectal temperature was recorded, using telethermometer (Electrolab, India), every 30 min after baclofen (10 mg/kg) till 180 min.

2.8.2. Baclofen-induced passivity

Rats were divided into groups of six each and administered baclofen (10 mg/kg) 30 min after BF, 25 mg/kg and passivity was scored every 30 min till 180 min as described by Turner (1972). In brief, rat was grasped with the thumb and index finger, which held the dorsal skin of the neck, while the rat was walking. The following scoring system was used: An unaffected rat escaped (score 0). The rat still grasped in the same manner held in vertical position, struggled (score 2), the unaffected rat when placed in supine position on the back of observers feast so that the thumb can support head of the rat, escaped (score 4), the unaffected rat tried to escape when held vertically by one forepaw (score 6) or by one hindpaw (score 8). Intermediate scores were used when struggle was diminished but not abolished.

Table 3
Effect of BF of *A. lebbeck* on baclofen-induced hypothermia in rats

	Rectal temperature						
Pretreatment (mg/kg)	0 min	30 min	60 min	90 min	120 min	150 min	180 min
Vehicle	35.6 ± 0.04	34.6 ± 0.4	33.5 ± 0.3	32.9 ± 0.3	33.06 ± 0.2	33.5 ± 0.9	33.9 ± 0.2
BF (25)	35.5 ± 0.09	35.0 ± 0.07	34.8 ± 0.2 *	34.6 ± 0.1 *	34.3 ± 0.2 *	34.5 ± 0.2 *	34.8 ± 0.2 *

$n = 5$; all values are means ± S.E.M.; baclofen (10 mg/kg ip) was administered 30 min after BF.
* $P < .05$ (Student's t test).

Table 4
Effect of BF of *A. lebbeck* on baclofen-induced passivity in rats

	Passivity (mean ± S.E.M.)						
Pretreatment (mg/kg)	0 min	30 min	60 min	90 min	120 min	150 min	180 min
Vehicle	0.0	3.1 ± 0.4	5.5 ± 0.7	7.3 ± 0.3	7.5 ± 0.4	6.8 ± 0.3	6.6 ± 0.3
BF (25)	0.0	1.3 ± 0.4 *	3.6 ± 0.6 *	4.0 ± 0.4 *	3.6 ± 0.2 *	3.5 ± 0.3 *	3.5 ± 0.3 *

$n = 6$; baclofen (10 mg/kg) was administered 30 min after BF.
* $P < .01$ vs. baclofen (Mann–Whitney U test).

2.8.3. Statistics

The observations are given as means ± S.E.M. The data was analyzed by one-way ANOVA followed by Student's t test. Baclofen-induced passivity was analyzed by Mann–Whitney U test. $P < .05$ was considered significant.

3. Results

3.1. Acute toxicity

The mice treated with the BF, 10 and 25 mg/kg orally, exhibited normal behavior, i.e. they were alert, with normal grooming, touch response, and pain response. There was no sign of passivity, stereotypy, and vocalization. Their motor activity and secretory signs were also normal. In higher doses, a dose-dependent depression of the central nervous system was noticed. The animals, which received 50 or 75 mg/kg, showed signs of depression. Alertness, motor activity, limb tone, and grip strength were diminished, and the animals showed staggering gait. In the animals treated with 100 mg/kg, the above signs of depression were more intense and 50% animals could not survive.

3.2. Test for neurotoxicity

Mice treated with lower doses of BF, i.e. 10 and 25 mg/kg ip remained on the rotating rod for complete duration of experiment, i.e. 3 min, whereas the animals treated with BF, 50 mg/kg, exhibited motor deficit and the fall off time was significantly ($P < .05$) reduced to 49.4 ± 3.5 s. The animals treated with diazepam fell down after 54.7 ± 5.7 s.

3.3. Nootropic activity

The retention of learned task was studied after 24 h as transfer latency on the elevated plus maze. The effect on transfer latency was expressed by IR. Increase in IR after 24 h indicated improved retention of learned task. Piracetam showed significant increase in IR compared to vehicle treated group and antagonized the effect of scopolamine, too. The BF in the doses of 10 and 25 mg/kg increased the IR and antagonized the effect of scopolamine $F(7,40) = 7.4$, $P < .05$] (Table 1).

3.4. Anxiolytic activity

The vehicle-treated mice spent 45.0 ± 3.2 s in open arm and 210.6 ± 10.9 s in closed arm with 2.2 ± 0.9 entries in open arm and 4.5 ± 2.4 entries into the enclosed arm. Diazepam (1 mg/kg) and BF (25 and 50 mg/kg) showed significant ($P < .001$) increase in the occupancy in open arm and decrease in occupancy in closed arm whereas BF, 10 mg/kg showed insignificant decrease in the time spent in closed arm. The animals treated with diazepam and BF showed decreased preference to the closed-arm entries and open-arm entries were significantly increased by all doses of BF. The observations are given in Table 2.

3.5. Baclofen-induced hypothermia

Baclofen produced fall in rectal temperature from 35.6 ± 0.04°C to 32.9 ± 0.3°C at 90 min. The peak hypothermic effect was observed 90 min after baclofen in the vehicle treated group. Prior treatment with BF (25 mg/kg) significantly ($P < .05$) inhibited the hypothermic activity of baclofen. The observations are given in Table 3.

3.6. Baclofen-induced passivity

Vehicle-treated animals showed absence of passivity as indicated by their struggle to escape when tried to grasp them. Baclofen reduced the tone of abdominal muscle and induced hypotonia. Passivity was noted within 30 min of its administration, and a time-dependent increase in passivity was observed in all the animals and peak effect was observed 120 min after baclofen, when the passivity score was 7.5 ± 0.4, i.e. most of the animals did not attempted to escape when they were hold by their hindleg. The prior administration of BF (25 mg/kg) significantly reduced the effect of baclofen on abdominal tone and hypotonia. The passivity was significantly less at every time of observation and the maximum score of 4.0 ± 0.4 (animals placed in supine position on the back of observers feast tried to escape) was observed after 90 min. The observations are given in Table 4.

4. Discussion

The study observed that the saponins containing fraction (BF) of leaves of *A. lebbeck* possessed nootropic and anxiolytic activity. This indicated dissociation between hypomnestic and anxiolytic activity as observed previously by Hasenohrl et al. (1998). The BF inhibited the $GABA_B$ mediated behavior as indicated by diminished hypothermic effect of baclofen in presence of BF. Ogasawara et al. (1999) has also reported such inhibition of the effect of baclofen. The higher dose of the BF (50 mg/kg) could not maintain equilibrium on the rotating rod suggesting neurological deficit and approximate oral median lethal dose was 100 mg/kg. This suggests that the BF has a narrow margin of safety.

The nootropic drugs belong to the category of psychotropic agents with selective facilitatory effect on intellectual performance, learning, and memory (Giurgea, 1973). The elevated plus maze is a widely accepted model to study nootropic activity (Itoh et al., 1990). The increase in the IR by BF per se proved that *A. lebbeck* possessed nootropic activity. Thus, the BF meets a major criterion for nootropic activity, i.e. improvement of memory in absence of cognitive deficit (Poschel, 1988). The BF in higher dose, i.e. 50 mg/kg, showed diminished nootropic activity, which may be due to sluggishness induced by the depressant activity associated with this dose. The inverse U dose–response relationship typical of these drugs (nootropic and anxiolytic agents) has been observed earlier (Pal et al., 1995; Hasenohrl et al., 1998). The antagonistic action of BF against scopolamine induced amnesia substantiates nootropic activity of BF.

The BF increased occupancy in the open arm of the elevated plus maze and also exhibited diminished preference to the closed arm. The anxiolytic activity was dose related. This finding is interesting with regards to the previous contention that anxiogenic agents can improve cognitive behavior and anxiolytics can impair learning and memory (Arolfo and Brioni, 1991; Cole and Jones, 1995; Janak and Martinez, 1992; Staubli and Xu, 1995).

Despite extensive experimental and clinical studies the neurochemical basis of learning and memory remains poorly understood. Although the role of central cholinergic system is fairly well established, the involvement of other neurotransmitter systems cannot be ignored (Hollander et al., 1986; Pepeu and Spignoli, 1989). It is well known that diazepam, a GABA mimetic induces memory impairment and the inhibition of $GABA_B$ receptor facilitates learning and memory (Olpe et al., 1993; Tsuji et al., 1996). Zhang et al. (1989) have reported that increase in cerebral GABA content is unfavorable to learning and memory. The antagonistic action of BF on baclofen-induced passivity and hypothermia, the behavior mediated by GABA, suggests that the observed effects on cognitive behavior and anxiolysis may be mediated via GABA. Sarter et al. (1992) have postulated that GABA antagonists may enhance cholinergic activity by blocking neurons that reach cholinergic nerve cells of basal forebrain.

Thus, it is concluded that the BF of petroleum ether extract of *A. lebbeck* leaves, which contained saponins, possessed nootropic and anxiolytic activity. The inhibition of GABAergic transmission may be responsible for the nootropic and anxiolytic activity of the saponins of *A. lebbeck*.

Acknowledgments

The authors are grateful to the All India Council for Technical Education, New Delhi for financial support.

References

Arnsten AF, Lin CH, Van Dyck CH, Stanhope KJ. The effects of 5-HT_3 receptor antagonists on cognitive performance in aged monkeys. Neurobiol Aging 1997;18:21–8.

Arolfo MP, Brioni JD. Diazepam impairs place learning in the morris water maze. Behav Neural Biol 1991;55:131–6.

Barness JM, Costall B, Coughlan J, Domeney AM, Gerrard PA, Kelly ME, Naylor RJ, Onaivi ES, Tomkins DM, Tyers MB. The effects of ondansetron, a 5-HT_3 receptor antagonist, on cognition in rodents and primates. Pharmacol, Biochem Behav 1990;35:955–62.

Blackburn TP, Baxter GS, Kennet GA, King FD, Piper DC, Sanger DJ, Thomas DR, Upton N, Wood MD. BRL 46470A: a highly potent, selective and long acting 5-HT_3 receptor antagonist with anxiolytic-like properties. Psychopharmacology (Berlin) 1993;110:257–64.

Cahill L, McGaugh JL. Amygdaloid complex lesions differentially affects retention of tasks using appetite and aversive reinforcement. Behav Neurosci 1990;104:532–42.

Chugh Y, Saha N, Sankarnarayanan A, Sharma PL. Memory enhancing effect of granisetron (BRL 43694) in a passive avoidance task. Eur J Pharmacol 1991;203:121–3.

Cole BJ, Jones GH. Double dissociation between the effects of muscarinic antagonists and benzodiazepine receptor agonists on the acquisition and retention of passive avoidance. Psychopharmacology (Berlin) 1995;118:37–41.

Costall B, Naylor RJ. Serotonin and psychiatric disorders. A key to new therapeutic approaches. Arzneimittelforschung 1992;42:246, 249.

Davis M. The role of amygdala in fear and anxiety. Annu Rev Neurosci 1992;15:353–75.

De Souza Silva M, Guimaraes FS, Graeff FG, Tomaz C. Absence of amnestic effects of an anxiolytic 5-HT_3 antagonist (BRL 46470) injected into basolateral amygdala, as opposed to diazepam. Behav Brain Res 1993;59:141–5.

Dunham NW, Miya TS. A note on a simple apparatus for detecting neurologial deficit in rat and mice. J Am Pharm Assoc Sci 1957;46:208–9.

Evans WC. Trease and Evans pharmacognosy. 14th ed. London: Hawoust Brace and Company Asia PTE, 1996. p. 293.

Gabryel B, Trzeciak HI. Nootropics: pharmacological properties and therapeutic use. Pol J Pharmacol 1994;46:383–94.

Giurgea C. The nootropic approach to the pharmacology of the integrative action of the brain. Cond Reflex 1973;8:108–15.

Hasenohrl RU, Topic B, Frisch C, Hacker R, Mattern CM, Huston JP. Dissociation between anxiolytic and hypomnestic effects for combined extracts of *Zingiber officinale* and *Ginkgo biloba*, as opposed to diazepam. Pharmacol, Biochem Behav 1998;59:527–35.

Hollander E, Mohs RC, Davis KS. Cholinergic approaches to the treatment of Alzheimer's disease. Br Med Bull 1986;42:97–100.

Itoh J, Nabeshima T, Kameyama T. Utility of an elevated plus maze for the

evaluation of memory in mice: effects of nootropics, scopolamine and electroconvulsive shock. Psychopharmacology 1990;101:27–33.

Jackson HC, Nutt DJ. Inhibition of baclofen-induced hyopthermia in mice by the novel $GABA_B$ antagonist CGP35348. Neuropharmacology 1991;30:535–8.

Jaffard R, Mocaer E, Ajaoul F, Beracochead D, Marighetto A, Meunier M. Effects de la tianepentine sur l'apprentissage et la memoire chez la souris. J Psychiat Biol Ther. 1989;37–9 (Edition Speciale).

Jaiswal AK, Bhattacharya SK. Effects of Shilajit on memory, anxiety and brain monoamines in rats. Indian J Pharmacol 1992;24:12–7.

Janak PH, Martinez JL. Cocaine and amphetamine facilitate retention of jump-up responding in rats. Pharmacol, Biochem Behav 1992;41:830–7.

Kulkarni SK, George B. Significance of long-term potentiation (LTP) in cognitive functions and epilepsy. Indian J Pharmacol 1999;31:14–22.

Lebrun C, Pilliere E, Lestage P. Effects of S18986-1, a novel cognitive enhancer, on memory performance in an object recognition task in rats. Eur J Pharmacol 2000;401:205–12.

Lee SC, Moon YS, You KH. Effect of red ginseng saponins and nootropic drugs on impaired acquisition of ethanol-treated rats in passive avoidance performance. J Ethnopharmacol 2000;69:1–8.

Missonnier P, Ragot R, Derouesne C, Guez D, Renault B. Automatic attentional shifts induced by a noradrenergic drug in Alzheimer's disease: evidence from evoked potentials. Int J Psychophysiol 1999;33:243–51.

Nadkarni AK. In: Nadkarni's Indian materia medica, vol. 1. Bombay: Popular Prakashan, 1996:296–666.

Nyback F, Wiesel A, Skett P. Effect of piracetam on brain monoamine metabolism and serum prolactin levels in the rat. Psychopharmacology 1979;61:235–8.

Ogasawara T, Itoh Y, Tamura M, Mushiroi T, Ukai Y, Kise M, Kimura K. Involvement of cholinergic and GABAergic systems in the reversal of memory disruption by NS-105, a cognition enhancer. Pharmacol, Biochem Behav 1999;64:41–52.

Olpe HE, Orner W, Saito H, Matsuki N. Stimulation parameters determine role of GABA receptors in long term potentiation. Experientia 1993; 49:542–6.

Pal BC, Achari B, Yoshikawa K, Arihara S. Saponins from *Albizzia lebbeck*. Phytochemistry 1995;38:1287–91.

Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus maze: a novel test for anxiety in the rat. Pharmacol, Biochem Behav 1986;24:525–9.

Pepeu G, Spignoli G. Nootropic drugs and brain cholinergic mechanisms. Prog Neuropsychopharmacol Biol Psychiatry 1989;13:S77–88.

Poschel BPH. In: Iversen LL, Iversen SD, Snyder SH, editors. Handbook of psychopharmacology, vol. 20. New York: Plenum, 1988. pp. 437–45.

Sarter M, Hagan J, Dudchenko P. Behavioral screening for cognition enhancers: from discriminate to valid testing. Psychopharmacology 1992;107:461–73.

Singh HK, Dhavan BN. Neuropharmacological effects of Ayurvedic nootropic *Bacopa monniera* Linn. Indian J Pharmacol 1997;29:359–65.

Staubli U, Xu FB. Effects of $5\text{-}HT_3$ receptor antagonism on hippocampal theta rhythm, memory and LTP induction in the freely moving rats. J Neurosci 1995;15:2445–52.

Tsuji M, Nakagawa Y, Ishibashi Y, Yoshii T, Takashima T, Shimada M, Suzuki T. Activation of ventral tagmental $GABA_B$ receptors inhibits morphine induced place preference in rats. Eur J Pharmacol 1996; 313:169–73.

Turner RA. Screening procedures in pharmacology. New York: Academic Press, 1972.

Venault P, Chapouthier G, Prado de Carvalho L, Simiand J, Morre M, Dodd RH, Rossier J. Benzodiazepines impairs and β-carboline enhances performance in learning and memory tasks. Nature 1986;321:864–6.

Yamada K, Noda Y, Nakayama S, Komori Y, Sugihara H, Hasegawa T, Nabeshima T. Role of nitric oxide in learning and memory and in monoamine metabolism in the rat brain. Br J Pharmacol 1995;115:852–8.

Ying Y, Zhang JT, Shi CZ, Liu Y. Study on the nootropic mechanism of ginsenoside Rb1 and Rb1-influene on mouse brain development. Acta Pharm Sin 1994;29:241–5.

Zhang SS, Zhu TJ, Zhang DS, Chen XY. Effects of cerebral GABA level on learning and memory. Acta Pharmacol Sin 1989;10:10–2.