ELSEVIER

Pharmacology, Biochemistry and Behavior 73 (2002) 893–900

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

A comparative study in rodents of standardized extracts of *Bacopa monniera* and *Ginkgo biloba* Anticholinesterase and cognitive enhancing activities

Amitava Das[a], Girja Shanker[a], Chandishwar Nath[a], Raghwendra Pal[b], Satyawan Singh[b], Hemant K. Singh[a,*]

[a]*Division of Pharmacology, Central Drug Research Institute, P.O. Box 173, Lucknow 226 001, India*
[b]*Division of Pharmaceutics, Central Drug Research Institute, P.O. Box 173, Lucknow 226 001, India*

Received 14 August 2001; received in revised form 14 February 2002; accepted 26 June 2002

Abstract

Bacopa monniera and *Ginkgo biloba* are well-known cognitive enhancers in Indian and Chinese traditional medicine systems. Standardized extracts of *B. monniera* and *G. biloba* were used to evaluate the antidementic and anticholinesterase activities in adult male Swiss mice. Antidementic activity was tested against scopolamine (3 mg/kg ip)-induced deficits in passive avoidance test. Three different extracts of *B. monniera* (30 mg/kg) and extract of *G. biloba* (15, 30 and 60 mg/kg) were administered postoperatively, daily for 7 days and 60 min after the last dose, i.e., on Day 7, first trial was conducted. In passive avoidance test, increased transfer latency time (TLT) and no transfer response (NTR) were taken as criteria for learning. TLT and NTR were significantly increased and decreased in second trial, 24 h after the first trial in control group and scopolamine-dementia group, respectively. The *B. monniera*- and *G. biloba*-treated groups produced significant increase in TLT and NTR on second trial (40–80%) after scopolamine treatment, thus, attenuating its antidementic effect. Both the extracts showed a dose (10–1000 μg)-dependent inhibitory effect on acetylcholinesterase (AChE) activity (in vitro), performed spectrophotometrically. IC_{50} of *G. biloba* was 268.33 μg, whereas none of the extracts of *B. monniera* showed more than 50% inhibition. At a dose concentration of 30 and 60 mg/kg, extracts of *G. biloba* showed a cognitive enhancing property and, at the same time, a significant decrease in AChE-specific activity in both per se and scopolamine-dementia groups. These extracts possess a significant anticholinesterase and antidementic properties, which may be useful in the treatment of dementia.

Keywords: *Bacopa monniera*; *Ginkgo biloba*; Cognition; Dementia; Acetylcholinesterase

1. Introduction

Bacopa monniera (Linn) Pennel [Syn: *Bacopa monniera* Wettst; *Gratiola monniera* (Linn); *Herpestis monniera* (Linn) Hb and K; *Moniera cuncifolia* Michx] (Family: Scrophulariaceae) is a perennial creeper found throughout India in wet, damp and marshy areas (Chopra et al., 1956). An infusion of the plant has been used in Indian folklore as a nerve tonic (Chunekar, 1960). In the ancient Indian system of medicine, viz., Ayurved, *B. monniera* has been classified under medicinal plants rejuvenating intellect and memory. Therefore, this plant has been investigated in several laboratories in India for its various neuropharmacological effects (Malhotra and Das, 1959; Aithal and Sirsi, 1961; Prakash and Sirsi, 1962).

The chemistry of the extracts of plant *B. monniera* was investigated in detail in our institute. The ethanolic extract was found to be a mixture of triterpenoids saponins designated as bacosides A and B (Chatterjee et al., 1963, 1965). Bacoside A comprised of a mixture of three saponins viz., Bacogenin A_1, A_2 and A_3, with A_3 being the major constituent (Kulshreshtha and Rastogi, 1973, 1974; Chandel et al., 1977). Its traditional memory-enhancing claim was established only when we reported the cognitive enhancing property of the alcoholic extract of *B. monniera* in several

* Corresponding author. Tel.: +91-522-212411x4391; fax: +91-522-223405.
E-mail addresses: amitavadascdri@rediffmail.com (A. Das), saritahemant@yahoo.co.in (H.K. Singh).

0091-3057/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0091-3057(02)00940-1

animal experimental models of learning from our laboratory (Singh and Dhawan, 1982, 1992, 1997; Singh et al., 1988).

Ginkgo biloba is one of the widely cultivated Chinese plants. The standardized extract of *G. biloba* has been shown to possess neuroprotective properties under conditions like hypoxia, ischemia, seizure activity and peripheral nerve damage (Smith et al., 1996). Recently, *G. biloba* has received attention as potential cognitive enhancer for the treatment of Alzheimer's disease (Oken et al., 1998; Mantle et al., 2000).

Inspite of great potential of *G. biloba* and *B. monniera* in the treatment of dementia disorders, effects of these plants on acetylcholinesterase (AChE) enzyme has not been investigated. Knowing the fact that the inhibition of AChE, the metabolizing enzyme of acetylcholine is presently the most accepted and recognized therapeutic marker for development of cognitive enhancers (Enz et al., 1993; Siddiqui and Levey, 1999), it becomes more pertinent to study the anticholinesterase activities of both these plants. Furthermore, a comparative evaluation of learning and memory enhancing properties of these plant extracts has also not been looked into. The aim of our present study was, therefore, to determine and compare the anticholinesterase and cognitive enhancing properties of the extracts of these two plants.

2. Methods

2.1. Chemicals

The biochemicals used in the study were acetylthiocholine iodide, 5,5′-dithio-bis (2-nitro benzoic acid) (DTNB), bovine serum albumin (BSA) and scopolamine hydrobromide (Sigma, USA).

2.2. Animals

Study was conducted on male Swiss mice (25–30 g). The animals were kept in polyacrylic cages (38 × 23 × 10 cm) with five animals per cage and maintained under standard housing condition (room temperature 24–27 °C and humidity 60–65%) with 12-h light and dark cycle. The food in form of dry pellets and water were available ad libitum.

The animal experiments were performed according to internationally followed ethical standards and approved by the research ethics committee of Central Drug Research Institute.

2.3. Drugs

The standardized extracts of *B. monniera* and *G. biloba* prepared within 3 months were used in the study. The shelf life of these extracts is 2 years.

The whole plant (including roots) of *B. monniera* was dried in shade and then powdered plant material was extracted with distilled water. The water extract was discarded and the residual plant material was extracted thrice with 90% ethanol. The residue obtained after removing the solvent was vacuum dried and macerated with acetone to give a free flowing powder. The extract of *B. monniera* contained 55–60% bacosides estimated according to the method of Pal and Sarin (1992). Three different extracts of *B. monniera* coded as I, II and III containing 20.1%, 23.2% and 36.9% of Bacogenin A_3, respectively, were extracted by the procedure described earlier (Chandel et al., 1977) and quantitatively determined by UV spectroscopy (Pal and Sarin, 1992) and HPLC (Pal et al., 1998).

The standardized extract of *G. biloba* (GINKOCER) was obtained from the Ranbaxy Laboratories, India. The preparation is a dried extract of *G. biloba* containing 24% of ginkgoflavonglycosides.

The extracts of *G. biloba* (15, 30 and 60 mg/kg) and the three extracts of *B. monniera* (30 mg/kg) were administered postoperatively, daily as aqueous suspension of gum acacia (0.5%) to different groups of mice ($n = 10$) for 7 days. After the completion of treatment, one subgroup ($n = 5$) was subjected to training and the another ($n = 5$) for per se effect of *G. biloba* on AChE activity (ex vivo) in the whole brain. Scopolamine (3 mg/kg ip) was used to induce retrograde dementia.

2.4. Training procedure: passive avoidance test

The mice were subjected to single trial passive avoidance test as described by Brioni et al. (1997). Briefly, an animal is placed in the lighted compartment of a computerized shuttle box (Columbus Instruments, OH, USA) provided with a software program PACS 30. An automated guillotine door isolated the compartment lighted at intensity of 8 (scale of 0—off and 10—brightest provided in the PACS 30 software) from the dark compartment. After an acclimatization period of 30 s, the guillotine door automatically opens and the animal is subjected to a trial of 270 s. An entry into the dark compartment automatically shuts the door and the subject is punished with a single low intensity foot shock (0.5 mA; 5 s). Infrared sensors monitor the transfer from one compartment to another, which is recorded as transfer latency time (TLT) in seconds. TLT was recorded in naive control, vehicle control, scopolamine-dementia and drug-treated group on Day 7 (first trial) and next day (second trial). The criterion for antidementic (cognitive) activity was taken as an increase in the TLT on second trial as compared to first trial. The number of mice not entering the darker compartment is expressed as percent no transfer response (NTR) for a group. Thus, TLT for mice showing NTR was recorded as 270 s, i.e., total period of observation. A retrograde dementia was induced by administering scopolamine (3 mg/kg ip) 5 min prior to first trial in all the extract treated as well as vehicle-treated control groups ($n = 5$). Another control was in the form of nonscopolamine-treated group ($n = 5$). The animals were exposed to the first trial 1 h after the last administration on Day 7.

2.5. AChE assay in brain

2.5.1. Tissue preparation

The mice were sacrificed by decapitation and the whole intact brain was removed carefully and placed on an ice-chilled petridish. Brain was washed with ice-chilled normal saline repeatedly to clean. A 10% (w/v) homogenate of brain samples was prepared first by homogenizing in an Ultra-Turrax T25 homogenizer at a speed of 9500 rpm thrice giving intervals for few seconds between the runs, with sodium phosphate buffer (30 mmol/l, pH 7.0). Sodium phosphate buffer was taken in a volume half to the final volume required for 10% homogenate.

One half volume of this homogenate was separated and used as salt soluble (SS) fraction for AChE assay and estimation of protein concentration in the samples.

1% Triton X-100 (1% w/v in 30 mmol/l sodium phosphate buffer, pH 7.0) was then added slowly while stirring the homogenate on ice, in sufficient volume to make the final volume for 10% homogenate. This fraction was used as detergent soluble (DS) fraction.

All the homogenates were centrifuged at 100,000 × *g* at 4 °C in Beckman Ultracentrifuge (LE 80), using a fixed angle rotor (80 Ti) for 60 min. Supernatant was collected and stored at 4 °C. Aliquots of this supernatant was diluted in the ratio of 1:10 and used as a source of enzyme for the assay.

2.5.2. Enzyme assay

The assay of AChE in the above mentioned supernatant was performed by modifying the method of Ellman et al. (1961) as described by Das et al. (2000) using acetylthiocholine iodide as substrate. A kinetic profile of the enzyme activity was studied spectrophotometrically at 412 nm at an interval of 15 s. The assay for each sample was run in duplicate and each experiment was performed thrice. The AChE activity is expressed as μmol/min/mg protein.

The effect of *B. monniera* and *G. biloba* extracts on AChE activity was studied in vitro at different concentration (10–1000 μg/50 μl) of extracts, incubated in the reaction mixture for 30 min at 37 °C. In the ex vivo set up, the *G. biloba* extracts were administered for 7 days in the dose schedule as described earlier and the animals were sacrificed 1 h after the last dose. AChE was assayed in the brain homogenate of these *G. biloba* extract treated mice as per method described earlier.

2.6. Protein assay

Protein was estimated in the brain samples by the methods of Lowry et al. (1951) and Wang and Smith (1975) in the SS and DS fractions, respectively. BSA was used as standard in the concentration of 1 mg/ml. and estimated in the range of 0.01–0.1 mg/ml.

2.7. Statistical analysis

Mean values and standard error (S.E.) of mean were calculated for specific activity of AChE in the brain regions and TLT in the passive avoidance test of each group. The data was subjected to nonparametric analysis by Mann–Whitney *U* test to determine statistical significance of difference between the values of first and second TLT obtained in different groups. NTR obtained in second trial

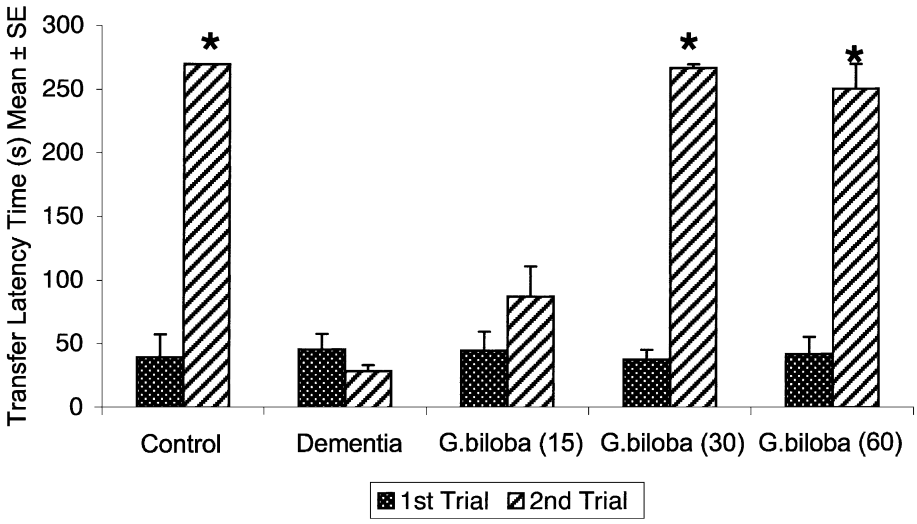

Fig. 1. Shows the antidementic effect of *G. biloba* extracts at 15, 30 and 60 mg/kg po on TLT in single trial passive avoidance test, significant difference from first trial, * $P < .001$. Scopolamine (3 mg/kg ip) 5 min prior to first trial in all the groups except control (vehicle-treated) to induce dementia (no significant increase in TLT on second trial).

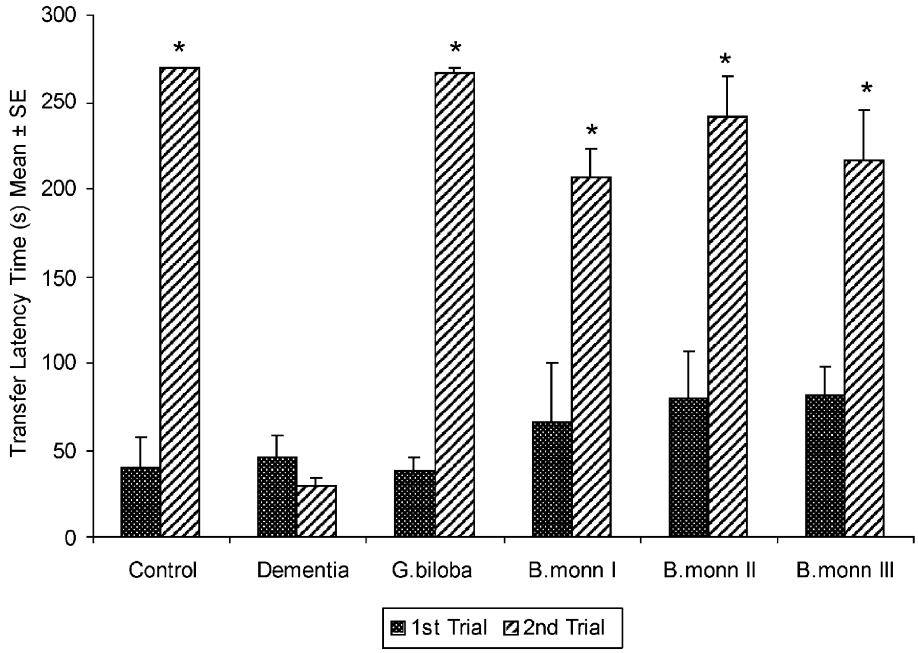

Fig. 2. Shows the comparative antidementic effect of *G. biloba* extract and three different extracts of *B. monniera* at 30 mg/kg po on TLT in single trial passive avoidance test, significant difference from first trial, $*P<.001$. Scopolamine (3 mg/kg ip) 5 min prior to first trial in all the groups except control (vehicle-treated) to induce dementia (no significant increase in TLT on second trial).

was analysed by test of proportion, Z test. Student's t test was employed to find out significance between the values of AChE activity of different groups.

3. Results

3.1. Passive avoidance task

In the naive control and vehicle control group, the mean ± S.E. TLT on second trial was 270 s, i.e., 100% no transfer (learning). In the scopolamine-treated (dementia) group, the TLT (mean ± S.E.) significantly decreased as compared to vehicle control, 28.8 ± 4.8 s on second trial ($P<.001$). The NTR was absent on second trial.

G. biloba-treated group also exhibited significant ($P<.001$) increase in TLT of 267 ± 3.0 s (30 mg/kg) and 250.4 ± 19.6 s (60 mg/kg) on second trial from first trial as compared to dementia group. The percentage NTR was increased from first trial (20%) to second trial (80%). The increase in TLT and NTR on second trial was significantly higher ($P<.001$) in *G. biloba* (30 and 60 mg/kg)-treated group as compared to scopolamine-treated (dementia) group. *G. biloba* treated at 15 mg/kg dose could not produce any significant learning as compared to scopolamine-treated (dementia) group (Fig. 1).

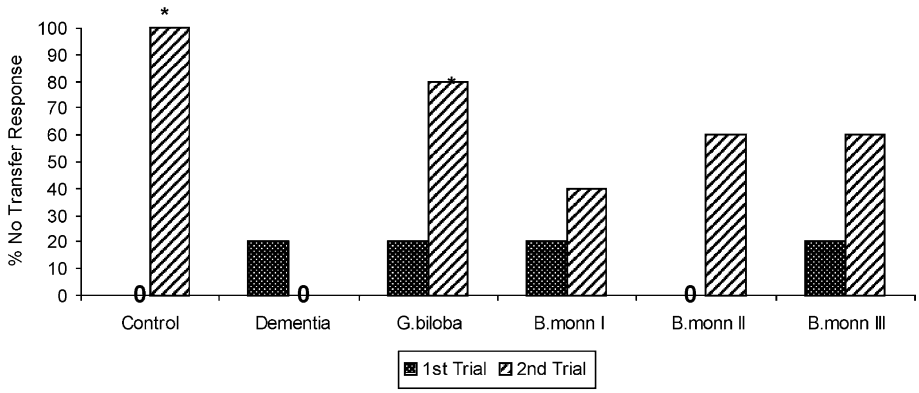

Fig. 3. Shows the comparative antidementic effect of *G. biloba* extract and three different extracts of *B. monniera* at 30 mg/kg po on the percent NTR parameter in single trial passive avoidance test, significant difference in second trial from dementia group, $*P<.05$. Dementia and control groups are the same as in Fig. 2.

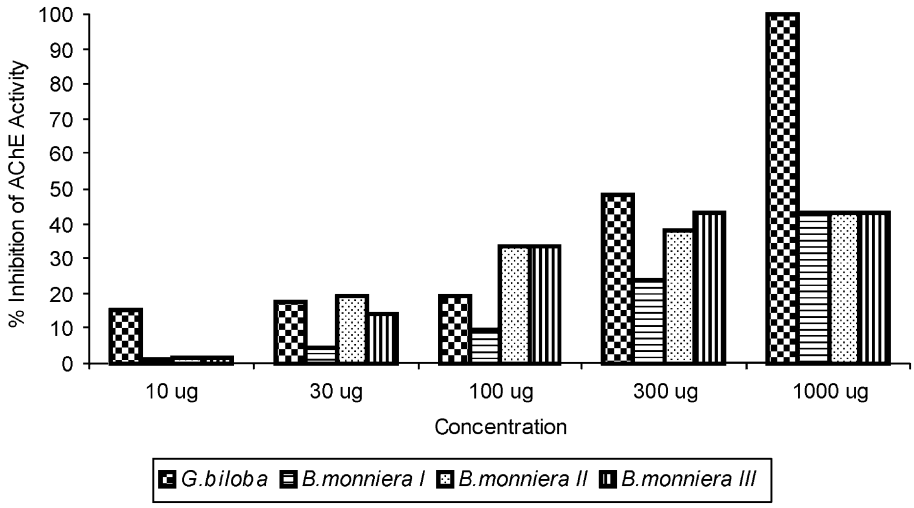

Fig. 4. Shows the effect of *G. biloba* extract and three different extracts of *B. monniera* on in vitro inhibition of AChE activity in mice brain.

For further comparative evaluation of *G. biloba* with *B. monniera*, only 30 mg/kg po dose was used.

All the extracts of *B. monniera* (30 mg/kg) showed highly significant increase ($P<.001$) in TLT, i.e., 207 ± 19.2, 241.4 ± 15.8, 216.4 ± 21.8 s, respectively, on second trial as compared to scopolamine-treated (dementia) group (Fig. 2). The respective percentage no transfers in *B. monniera* extracts I, II and III treated group were from 20%, 0% and 20% on first trial to 40%, 60% and 60% on second trial, respectively (Fig. 3).

3.2. Anticholinesterase activity in mice brain

3.2.1. Anticholinesterase activity (in vitro) in mice brain

B. monniera and *G. biloba* extracts showed a concentration dependent inhibition of AChE activity. The AChE was inhibited more in presence of *G. biloba* than *B. monniera* as compared to control in a reaction volume of 3 ml. *G. biloba* showed 15.2 ± 1.84%, 17.4 ± 1.06%, 19.6 ± 2.9%, 48.4 ± 3.7% and 100 ± 0.0% inhibition at 10, 30, 100, 300 and 1000 μg concentrations, respectively. *B. monniera* extracts I, II and III showed 1.0 ± 0.3%, 1.5 ± 0.6% and 1.5 ± 0.6% at 10 μg, 4.8 ± 1.0%, 19.1 ± 3.6% and 14.3 ± 3.2% at 30 μg, 9.5 ± 1.5%, 33.3 ± 2.04% and 34.4 ± 2.25% at 100 μg, 23.8 ± 1.4%, 38.1 ± 1.9% and 42.9 ± 3.4% at 300 μg and 37.8 ± 3.4%, 42.9 ± 3.4% and 42.9 ± 1.2% at 1000 μg, respectively (Fig. 4). IC_{50} for the inhibition AChE activity in vitro by *G. biloba* was 268.33 μg. whereas none of the extracts of *B. monniera* showed more than 50% inhibition indicating a different mechanism of action. Therefore, ex vivo studies were not carried out with these extracts of *B. monniera*.

The same in the SS fraction did not show any significant inhibitory effect (data not shown).

3.2.2. Anticholinesterase activity (ex vivo) in brain of G. biloba-treated mice

Groups of mice treated with extracts of *G. biloba* at different concentrations of doses were sacrificed after 1 h of the last dose and the tissue preparation was performed as described earlier. AChE was assayed in the samples to find out the per se effect of this extract. There was a significant

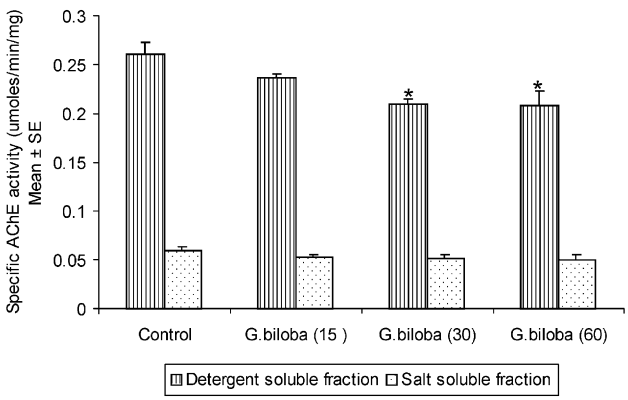

Fig. 5. Shows the per se effect of *G. biloba* extracts on ex vivo AChE activity in mice brain. The AChE activity for each group denotes mean ± S.E. values of six observations, * $P<.01$ significant difference from control group.

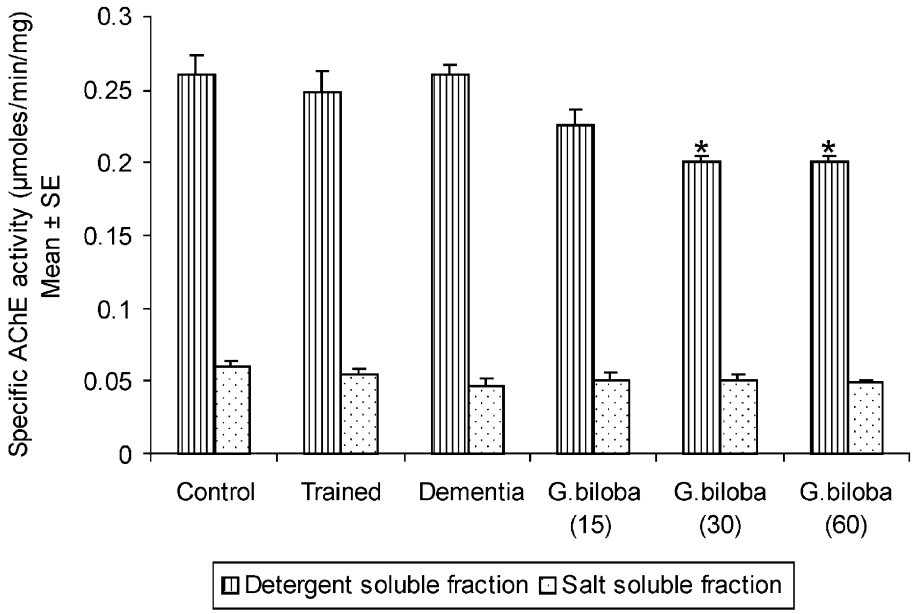

Fig. 6. Shows the effect of *G. biloba* extracts on ex vivo AChE activity in mice brain, in scopolamine-induced dementia model. The AChE activity for each group denotes mean ± S.E. values of six observations, * $P < .001$ significant difference from control, control-trained and scopolamine-dementia group. Control-trained group did not receive any drug.

decrease in AChE-specific activity (mean ± S.E.) at 30 mg/kg (0.20932 ± 0.005) and 60 mg/kg (0.20848 ± 0.014) as compared to vehicle control (0.26018 ± 0.013) in the DS fraction. However, no significant change was observed at 15 mg/kg treated group. No significant difference was seen in the SS fraction in any of the treated as well as control groups (Fig. 5).

3.2.3. Anticholinesterase activity (ex vivo) in brain of G. biloba-treated dementia group

No significant difference in AChE specific activity was observed in both DS and SS fractions of naive control, vehicle control, control-trained and dementia groups. The AChE-specific activity was significantly decreased in the DS fraction of 30 and 60 mg/kg *G. biloba*-treated dementia groups, 0.2004 ± 0.004 and 0.20104 ± 0.004, respectively, from dementia (0.25977 ± 0.007), naive control (0.26122 ± 0.012), vehicle control (0.26018 ± 0.013) and control-trained (0.24862 ± 0.014) groups. No changes were observed in SS fraction of 30 and 60 mg/kg and both the DS and SS fractions of 15 mg/kg *G. biloba*-treated dementia group (Fig. 6).

4. Discussion

Central cholinergic system is considered as the most important neurotransmitter involved in regulation of cognitive functions. Cholinergic neuronal loss in hippocampal area is the major feature of Alzheimer's disease and enhancement of central cholinergic activity by use of anticholinesterase, is presently the mainstay of the pharmacotherapy of senile dementia of Alzheimer type (Enz et al., 1993; Siddiqui and Levey, 1999).

Anticholinergics induced transient impairment in memory in a passive avoidance test is widely employed to bioevaluate learning and memory performance. In the present study, scopolamine-treated mice showed decreased TLT on second trial, indicating deficit in passive avoidance (dementia). The *B. monniera* and *G. biloba* extracts treated groups showed a significant increase in TLT on second trial as compared to dementia group. Attenuation of scopolamine-induced deficits in passive avoidance clearly indicates the potential of these extracts as cognitive enhancer. All the three extracts of *B. monniera* showed a similar profile against scopolamine-dementia, which suggests the equipotency of Bacogenin A_3 in the range of 20.1–36.9%. The data on passive avoidance experiments may further be analysed qualitatively if we consider NTR (no transfer into darker chamber on second trial) as an indicator of complete learning. Then, *G. biloba* extracts apparently induced more learning than the *B. monniera* extracts as is evident from the NTR of mice with 80% in the former treated group and 40–60% in the later treated group. But this can also be attributed to the suppression of fear induced by *B. monniera* extracts because of their mild anxiolytic effect (Shanker and Singh, 2000). The *G. biloba* extract has not been reported to possess any anxiolytic property.

Cognitive enhancement is an important addition to neuroprotective properties of *G. biloba*. The neuroprotective property of *G. biloba* leaf has been shown to be because of ginkgolide B, a terpene fraction but its flavanoid fraction containing free radical scavengers is also important (Smith et al., 1996; Perry et al., 1998).

Some workers have shown the cognitive enhancing property of standardized extract of *G. biloba* (Egb 761) and attributed it to serotonergic mechanism—5-HT_{1A} receptors (Bolanos-Jimenez et al., 1995; Carli et al., 1995). But the effect of *G. biloba* extract on cholinergic system particularly AChE activity has not been reported so far.

In this study, significant inhibition of AChE activity in the brain was obtained in vitro as well as ex vivo by *G. biloba* extract but not by *B. monniera* as compared to the controls and dementia group. It is well documented that the AChE enzyme occurs in different molecular isoforms having differential localization in neuronal cell (Adamson, 1977). The two major isoforms are globular monomer (G1) protein and globular tetramer (G4) of the same monomer subunit. The G1 isoform is reported to be present in the soluble cytoplasm of the neuronal cells whereas the G4 isoform is predominantly a membrane bound enzyme (Massoulie et al., 1993). The experimental procedure of Sberna et al. (1998) was followed in the present study that leads to extraction of different isoforms in different fractions, i.e., the SS fraction consists of mainly the G1 form of AChE, whereas the DS fraction contains predominantly the G4 form of AChE.

Anti-AChE activity of *G. biloba* was more pronounced in DS fraction (G4), whereas there was no significant change in the SS fraction (G1) in vitro and ex vivo experiments.

The doses of *G. biloba* (30 and 60 mg/kg) which inhibited the AChE activity in both the setups of ex vivo experiments—per se as well as dementia were effective against scopolamine-induced dementia. Moreover the non-inhibitory dose of *G. biloba* (15 mg/kg) against AChE was ineffective in reversing the dementia. Therefore, the inhibition of AChE activity can be correlated with improvement observed in scopolamine-induced deficits in passive avoidance by *G. biloba* extract. The decrease in AChE activity indicates an increase in the basal level of acetylcholine, which might be helpful in maintaining the learning and memory functions in the dementia group. Since DS (G4) but not the SS form is affected by *G. biloba*, it appears that the G4 isoform of AChE is responsible for maintaining the learning and memory functions.

In conclusion, the extracts of *G. biloba* and *B. monniera* have potent cognitive enhancing property but with different mechanisms of action. On the basis of inhibition of AChE, it can be suggested that improvement of perturbed cholinergic function might be an important contributory factor for the cognitive enhancing property of *G. biloba* extracts.

Acknowledgements

We express our gratefulness to Ranbaxy Laboratories, India for providing the *G. biloba* extracts. Dr. S.K. Mandal, Division of Biometry deserves our special thanks for statistical analysis. One of the authors (AD) is grateful to CSIR (India) for providing Senior Research Fellowship.

References

Adamson ED. Acetylcholinestrase in mouse, erythrocytes and muscles. J Neurobiochem 1977;8:605–54.

Aithal HN, Sirsi M. Pharmacological investigation on *Herpestis monniera*. Ind J Pharmacol 1961;23:2–5.

Bolanos-Jimenez F, de Castro RM, Sarhan H, Prudhomme N, Drieu K, Fillion G. Stress-induced 5-HT_{1A} receptor desensitization: protective effects of *Ginkgo biloba* extract (Egb 761). Fundam Clin Pharmacol 1995;9:169–74.

Brioni JD, Hock FJ, McGaugh JJ. Drug effects on learning and memory. In: Vogel GH, Vogel WH, editors. Drug discovery and evaluation: pharmacological assays. New York: Springer, 1997. p. 335–6.

Carli M, Luschi R, Samanin R. [S]-WAY 100135; a 5-HT_{1A} receptor antagonist; prevents the impairment of spatial learning caused by intrahippocampal scopolamine. Eur J Pharmacol 1995;283:133–9.

Chandel RS, Kulshreshtha DK, Rastogi RP. Bacogenin A_3: a new sapogenin from *Bacopa monniera*. Phytochemistry 1977;16:141–3.

Chatterjee N, Rastogi RP, Dhar ML. Chemical examination of *Bacopa monniera* Wettst: Part I. Isolation of chemical constituents. Ind J Chem 1963;1:212–5.

Chatterjee N, Rastogi RP, Dhar ML. Chemical examination of *Bacopa monniera* Wettst: Part II. The constitution of bacoside A. Ind J Chem 1965;3:24–9.

Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. New Delhi: Council of Scientific and Industrial Research, 1956. p. 32.

Chunekar KC. *Bhav Prakasa Nighantu*. Varanasi: Hindi Translation, 1960. p. 372.

Das A, Kapoor K, Sayeepriyadarshini AT, Dikshit M, Palit G, Nath C. Immobilization stress-induced changes in acetylcholinesterase activity and cognitive function in mice. Pharmacol Res 2000;43:213–7.

Ellman GE, Courtney KD, Andersen V, Featherstone RM. A new and rapid calorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88–95.

Enz A, Amstutz R, Boddeke H, Gmelin G, Malanowski J. Brain selective inhibition of acetylcholinesterase: a novel approach to therapy for Alzheimer's disease. Prog Brain Res 1993;98:431–8.

Kulshreshtha DK, Rastogi RP. Bacogenin A_1: a novel dammerane triterpene sapogenin from *Bacopa monniera*. Phytochemistry 1973;12:887–92.

Kulshreshtha DK, Rastogi RP. Bacogenin A_2: a new sapogenin from bacosides. Phytochemistry 1974;13:1205–6.

Lowry OH, Rosebrough NJ, Farr AI, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.

Malhotra CL, Das PK. Pharmacological studies of *Herpestis monniera* Linn (Brahmi). Ind J Med Res 1959;47:294–305.

Mantle D, Pickerins AT, Perry EK. Medicinal plant extract for the treatment of dementia: a review of their pharmacology, efficacy and tolerability. CNS Drugs 2000;13:201–13.

Massoulie J, Pezzementi I, Bon S, Krejci E, Vallette FM. The molecular and cellular biology of cholinesterases. Prog Neurobiol 1993;41:31–91.

Oken BS, Storzbach DM, Kaye JA. The efficacy of *Ginkgo biloba* on cognitive function in Alzheimer disease. Arch Neurol 1998;55:1409–15.

Pal R, Sarin JPS. Quantitative determination of bacosides by UV-spectrophotometry. Ind J Pharmacol Sci 1992;54:17–8.

Pal R, Dwivedi AK, Singh S, Kulshreshtha DK. Quantitative determination of bacosides by HPLC. Ind J Pharmacol Sci 1998;60:328–9.

Perry EK, Pickering AT, Wang WW, Houghton P, Perry NS. Medicinal plants and Alzheimer's disease: integrating ethnobotanical and contemporary scientific evidence. J Altern Complement Med (US) 1998;4:419–28.

Prakash JC, Sirsi M. Comparative study of the effects of Brahmi (*Bacopa monniera*) and Chlopromazine on motor learning in rats. J Sci Ind Res 1962;21:93–6.

Sberna G, Valero JS, Li QX, Czech C, Beyreuther K, Masters CL, Mclean CA, Small DH. Acetylcholinesterase is increased in the brains of transgenic mice exposing the C-terminal fragment (CT 100) of the β-amyloid protein precursor of Alzheimer's disease. J Neurochem 1998;71:723–31.

Shanker G, Singh HK. Anxiolytic profile of standardized Brahmi extract. Ind J Pharmacol 2000;32:152.

Siddiqui MF, Levey AI. Cholinergic therapies in Alzheimer's disease. Drugs Future 1999;24:417–44.

Singh HK, Dhawan BN. Effect of *Bacopa monniera* (Brahmi) extract on avoidance responses in rats. J Ethnopharmacol 1982;5:205–14.

Singh HK, Dhawan BN. Drugs affecting learning and memory. In: Tandon PN, Bijlani V, Wadhwa S, editors. Lectures in neurobiology. New Delhi: Wiley; 1992. p. 189–207.

Singh HK, Dhawan BN. Neuropsychopharmacological effects of the Ayurvedic Nootropic *Bacopa monniera* Linn (Brahmi). Ind J Pharmacol 1997;29:S359–65.

Singh HK, Rastogi RP, Srimal RC, Dhawan BN. Effect of bacosides A and B on avoidance responses in rats. Phytother Res 1988;2:70–5.

Smith PF, Maclennan K, Darlington CL. The neuroprotective properties of the *Gingko biloba* leaf: a review of the possible relationship to platelet-activating factor (PAF). J Ethnopharmacol 1996;50:131–9.

Wang CH, Smith RL. Lowry determination of protein in the presence of triton X-100. Anal Biochem 1975;63:414–7.