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Neuroprotective effect of *Bacopa monniera* on ischemia induced brain injury

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ABSTRACT

Purpose: Brain stroke is a leading cause of death without effective treatment. *B. monniera*, an Indian herbal medicine, exerts antioxidant activity and antistress activity by modulating the antioxidative defence system. We wanted to test if *B. monniera* could alleviate the ischemia induced brain injury and cognitive dysfunction in Wistar rats.

Procedure: We studied the effect of *B. monniera* (120 mg kg^{-1}, 160 mg kg^{-1} and 240 mg kg^{-1} P.O.) on transient intracarotid artery (ICA) occlusion induced ischemia by testing the neurobehavioral and biochemical parameters on treated and control rats.

Findings: B. monniera attenuated the reduced transfer latency in ischemic rats in a step through test and showed a protective effect on ischemia induced memory impairment in the plus maze task. It also showed a marginal improvement in neurodeficit score and fore limb muscle grip strength. *B. monniera* reduced the infarct size in the ischemic brain. It also decreased nitrite, nitrate and lipid peroxidation and significantly improved catalase activity.

Conclusion: These observations suggest the neuroprotective and antioxidant activity of *B. monniera* on ischemia induced brain injury and pave the way for future investigations.

1. Introduction

Stroke is one of the leading causes of death worldwide (Rensink et al., 2009). World Health Organization (WHO) data reflects 5.7 million deaths from cerebrovascular diseases out of 58 million global deaths in 2005. The number of people with transient ischemic attack (TIA) is estimated to be even greater. There are studies which indicate that the incidence of second stroke is higher in survivors of TIA (Sacco et al., 2006). Brain stroke is a sudden loss of brain function usually caused by a blockade or leakage of a blood vessel. It develops from a complex cascade of cellular events that ultimately leads to cerebral infarction (Hou and MacManus, 2002) and causes sudden loss of vision, balance, coordination, speech and memory (O'Brien et al., 2003). Severe strokes may also lead to sudden death.

Reactive oxygen species (ROS) are formed during cerebral ischemia due to calcium overload-induced inhibition of mitochondrial electron transport chain and activation of phospholipase A2 (Moskowitz et al., 1984). Reperfusion of the ischemic brain may further aggravate neuronal injury by increasing free radical formation (del Zoppo and Hallenbeck, 2000). Moreover, free radicals may also promote lipid peroxidation (Kumar and Gupta, 2002) which ultimately alters the integrity of the plasma membrane. ROS such as peroxynitrite (Epe et al., 1996) and hydroxyl radical (Delaney et al., 1997) are reported to produce DNA nicking. ROS are also documented to activate lysosomal enzymes, which may contribute to neuronal injury (Ollinger and Brunk, 1995). Additionally, mitochondrial damages due to ROS release may contribute to delayed cell death after cerebral ischemia and reperfusion (Fiskum, 2000).

Bacopa monniera, an Indian herbal drug (Syn. Brahmi) displays antioxidant (Tripathi et al., 1996), antistress (Chowdhuri et al., 2002) and anxiolytic (Shanker and Singh, 2000; Singh and Singh, 1980) activities. It has also been shown to exert antioxidant effects through chelating of metal ions, breaking oxidative chain reaction (Tripathi et al., 1996), improving activities of antioxidative defence enzymes (Bhattacharya et al., 2000a) and scavenging of free radicals (Russo et al., 2003). It exhibits an antistress activity in rat by modulating the activities of Hsp70, P450 and SOD (Chowdhuri et al., 2002), repairing the damaged neurons by enhanced kinase activity, neuronal synthesis coupled with restoration of synaptic activity and nerve impulse transmission (Kishore and Singh, 2005; Singh and Dhawan, 1997), which could be partly responsible for its cognition facilitation activity. The memory enhancing effects have been attributed to the active constituent bacosides A (Chatterji et al., 1965) and B (Dhawan and Singh, 2002). This facilitates the effect on retention capacity in avoidance response in rats (Singh et al., 1988) and ability to reverse amnesic effects of neurotoxin, scopolamine and electric shock (Bhattacharya et al., 2000b).

Therapeutic agents such as tissue plasminogen activator, antioxidants, and glutamate receptor blockers show limited efficacy in

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stroke and therefore a number of studies have been initiated for the improvement of therapeutic strategies of brain stroke. Several herbal drugs have also shown a promising neuroprotective effect in rodents (Chen et al., 2003; Kim et al., 2008; Lee et al., 2003; Sun et al., 2008; Tian et al., 2008; Zhou et al., 2009). There are some herbal drugs which have shown a dual role of exerting antiamnesic and neuroprotective effects (Kim et al., 2008; Lin et al., 2005; Siripurapu et al., 2005; Wang et al., 2005, 2008; Yun et al., 2007). *B. monniera* is also reported to provide neuroprotection against cigarette smoke induced apoptosis (Anbarasi et al., 2005a,b,c, 2006a,b) and aluminium induced oxidative stress (Jyoti et al., 2007). In addition, our own earlier investigations have revealed antiamnesic effects of *B. monniera* using several amnesic agents. Therefore, in this study we wanted to expand our investigation with this drug and explore its effect on ischemia induced brain injury by studying its association with behavioral parameters.

2. Materials and methods

2.1. Materials and subjects

Male Wistar rats weighing 200–250 g were used in the present study. They were housed individually with *ad labium* access to food and water under controlled laboratory conditions and were exposed to a 12 h cycle of light and dark. The experiments were conducted in a semi-sound proof laboratory. The dried powder of *B. monniera* (Brahmi) standardized extract, containing 55.34% of bacosides, was obtained from Lumen Marketing Company, Chennai. The suspension of *B. monniera* extract was prepared in 5% w/v Tween 80. Sodium selenite was prepared in normal saline.

2.2. Transient global ischemia

All experiments were performed in accordance with the guidelines of the institute animal ethical committee. Adequate measures were taken to minimize pain or discomfort with animal experimental procedures. Rat was anaesthetized with ketamine (50 mg/ml) and xylazine (50 mg/ml) cocktail (9:1) injected intra-peritoneally combined with analgesic (diclofenac sodium). A small cut was made on the neck region and the neck muscle was retracted for isolation of the common carotid artery. The internal carotid artery was subsequently isolated. 30 min of ischemia was given to the rat by blocking the internal carotid artery with a micro-vascular clip. After the ischemic period, the neck muscle was tied and an antibiotic was applied. The test drug solution or suspension was administered to the respective group of rats described below for a period of seven days. Group 1 (sham) rats were injected with vehicle (5% Tween 80 10 ml kg^{-1} orally). The ischemia was produced in groups 2 to 6. After the induction of 30 min ischemia, group 2 rats were administered with vehicle (5% Tween 80 10 ml kg^{-1} orally for 8 days). Sodium selenite (2 mg kg^{-1} i.p.) was injected in group 3. *B. monniea* at 120 mg kg^{-1}, 160 mg kg^{-1} and 240 mg kg^{-1} was administered orally for 8 days to ischemic rats of groups 4 to 6 respectively. 7 additional days of post ischemic survival time was provided. On the 7th and 8th days behavioral studies were carried out. Rats were sacrificed by an overdose of anesthesia on the 8th day after completion of behavioral tests. The isolated brains were frozen for TTC staining and biochemical tests.

2.3. Neurobehavioral test

2.3.1. Neurodeficit score

The neurological status of the animals was evaluated using the methods described by Bederson et al. (1986). Accordingly, four categories of neurological findings were noted: 0 = no observed neurological deficit; 1 = contralateral forelimb flexion with wrist flexion and shoulder adduction; 2 = reduced resistance to lateral push; and 3 = circling movements towards the ipsilateral side.

2.3.1.1. Rota rod test. Sensorimotor performance was evaluated using a rota rod test. All animals were tested for their ability to remain on the rotating bar at a speed of 14 rpm. Each animal was provided a minimum of three trials. After 8 post ischemic days the animals were tested for motor impairment after administration of test drugs. Latency to fall off the rotating rod was noted for each trial with a 5 min maximum to termination of the trials.

2.3.2. Hanging wire

The experimental animals were suspended by its forelimbs on a wire stretched between 2 posts, 45 cm above a foam sheet. The time (in seconds), until the animal fell down, was recorded. 2 min of cut off time was designated. This task was used as a measure of grasping ability and forelimb strength.

2.3.3. Pole fall

The rat was placed in a pole of 1 inch diameter 45 cm above a foam sheet. The time (in seconds) until the animal fell down was recorded. A maximum of 2 min was designated as cut off time. This task was used as a measure of grasping ability and forelimb strength.

2.4. Memory test

2.4.1. Elevated plus maze

Plus maze for rat consists of a central platform connected to two open arms and two enclosed arms. The maze is elevated to a height of 50 cm from the floor. During training trials the animal was placed at the end of an open arm, facing away from the central platform of the maze. The time taken by the animal to move from open arm and cross the line marked in enclosed arm with all four paws was recorded as transfer latency time (TL). In case the rats did not enter the enclosed arm within 90 s, it was gently pushed into the enclosed arm and a TL of 90 s was assigned to it. The animal was allowed to remain in the maze for the duration of 10 s. The TL measured on the plus maze on the first day serves as an index of acquisition, whereas the TL measured after 24 h of acquisition trial was taken as an index of retrieval.

2.4.2. Passive avoidance paradigm

The tendency of rodents to avoid bright light and prefer a darker side is the principle of this technique. The test apparatus consists of a small illuminated chamber connected to a large dark chamber via a door. The latency time to enter the dark compartment from the light compartment within 180 s was noted as an index of acquisition. Once the animal enters into the dark chamber, a brief electric foot shock was delivered. After 2 and 24 h the retention test was performed and the time taken by the animal to enter the dark compartment from the illuminated compartment was noted. Prolongation of this step through latency time indicated acquisition and retrieval of the memory for the foot shock received by the animal.

2.5. Cerebral infarct size

Frozen brain was sectioned into uniform slices of about 2 mm thickness. The slices were incubated in 2% TTC (triphenyl tetrazolium chloride) solution at 37 °C in a PBS buffer for 10 min. The slices were preserved in 10% normal formal saline solution. The slices were arranged in a glass plate in a row and the images were captured. We used software Image J (Version 3.00, University of Texas Health Science Center, San Antonio) for measurement of the area of infarction (yellow area) and the total area of the section (pink area + yellow area). The percentage of the area of infarction was calculated as [(yellow area/total area) × 100].

2.6. Biochemical investigation

We used 8 rats per group for behavioral tests. After completion of the behavioral test, the brain was isolated. At least 3 brains per group were used for histological studies and the remaining 5 brains were used for biochemical tests. We did not pool the brain for biochemical tests. The small amount of a homogenized sample of the individual brain was aliquoted in various tubes and used for various biochemical tests.

2.6.1. Nitric oxide estimation

The brain was analyzed for nitric oxide, SOD, GPx, catalase and lipid peroxidase assay. Nitric oxide was estimated by an indirect measurement of nitrite, nitrate and total nitrite in rat brain extract supernatants obtained after centrifugation. Cadmium beads were added to the supernatant for total nitrite estimation and incubated overnight. The clear supernatant was treated with a mixture of coloring reagents I (1% sulfanilamide in 3 N HCl) and II (0.1% NED·2 HCl) and incubated for 10 min at room temperature. The absorbance was noted at 620 nm and 550 nm respectively. The nitrite and total nitrite level were normalized to the total protein estimated by Bradford method.

2.6.2. SOD estimation

SOD level was measured by superoxide dismutase estimation kit (Sigma, USA) in brain homogenates. The protocol was adapted to kit instructions and was normalized by total protein. Total protein was estimated by Bradford method.

2.6.3. GPx estimation

The GPx level was measured by glutathioperoxidase estimation kit (Sigma) in brain homogenates. The standard protocol as described in the kit was followed. The GPx level was estimated, which was normalized by total protein. The total protein was estimated by Bradford method.

2.6.4. Lipid peroxidase estimation

The lipid peroxidation in the homogenate was determined by the method of Wills (1966). The tissue homogenate with 0.1 M of Tris–HCl buffer was incubated at 37 °C for 2 h. After incubation 10% w/v ice cold TCA was added. The reaction mixture was centrifuged at 8000 *g* for 10 min. 0.67% TBA was added to the supernatant and the tubes were kept in a boiling water bath for 10 min till the pink color appeared. The absorbance was measured at 550 nm and the amount of malondialdehyde content was calculated using the molar extinction coefficient of MDA-TBA chromophore ($1.56\times10^5\ M^{-1}\ cm^{-1}$). The results were expressed as nmol of MDA/mg protein.

2.6.5. Catalase estimation

Catalase activity was assayed in the postmitochondrial supernatant by the method of Luck (1971). An appropriate amount of sample was added to the cuvette containing hydrogen peroxide phosphate buffer. The contents were mixed thoroughly. The decrease in absorbance was followed at 240 nm for 3 min at a 30 s interval. The activity of enzyme was expressed as μmol of H_2O_2 decomposed/min/mg protein, using the molar extinction coefficient of H_2O_2 ($0.71\ M^{-1}\ cm^{-1}$).

2.7. Statistical analysis

Behavioral data was analyzed by ANOVA followed by post hoc 'least significant difference' (LSD). '*' indicates a significant difference for treated group vs control group at $p<0.05$. '$' indicates a significant difference for treated group vs ischemic group at $p<0.05$. Biochemical data were analyzed by ANOVA followed by least significant difference (LSD). 'a' indicates a significant difference for treated group vs control group at $p<0.05$. 'b' indicates a significant difference for treated group vs ischemic group at $p<0.05$.

3. Results

The rat model of ischemia was established by temporary blocking of the internal carotid artery for 10 min, 30 min, 60 min and 120 min and assessing the cognitive and neurological status (data not shown). We then chose to extend our investigations with 30 min ischemia as the investigative measure as it creates infarction and cognitive impairment without severe paralytic symptoms.

3.1. B. monniera attenuates ischemia induced memory deficits

We tested the effect of different doses of *B. monniera* at 120 mg, 160 mg and 240 mg kg^{-1} orally and sodium selenite 2 mg kg^{-1} intraperitoneally for 8 days in ischemic rats. There was a reduction in the transfer latency in ischemic animals using a step through test which was attenuated by *B. monniera* and sodium selenite treatment (Fig. 1A). In the plus maze task, *B. monniera* showed more protective effects as compared to sodium selenite on ischemia induced impairment in the retrieval of memory (Fig. 1B).

3.2. B. monniera improved neurological score and reduced infarct size in ischemic rat

Neurobehavioral tests such as neurodeficit scoring, pole fall and hanging wire results showed impairment in neurobehavioral scale using ischemic rats (Table 1). Some of the neurobehavioral outcomes (such as neurodeficit score and fore limb muscle grip strength) of these rats showed a moderate improvement by *B. monniera* treatment while muscle grip strength measured by pole fall remained unaffected in the *B. monniera* treated group. We also observed that *B. monniera* and sodium selenite reduced the infarct size measured by TTC staining of frozen brain section of the rat (Fig. 2A).

3.3. B. monniera exerts antioxidant effect in ischemic rat

The total nitrite was elevated in ischemic rats. Sodium selenium treatment and *B. monniera* treatment reduced the enhanced total nitrite in ischemic rat as compared to untreated ischemic rat (Fig. 2B). Similar to sodium selenite, *B. monniera* also reduced the lipid peroxidation in ischemic rat as compared to untreated ischemic rats (Fig. 2C). The catalase activity was significantly enhanced in selenite and *B. monniera* (240 mg kg^{-1} orally) co-treated rats while the lower dose of *B. monniera* (120 mg kg^{-1} and 160 mg kg^{-1} orally) did not affect the catalase activity as compared to controls (Fig. 2D). The superoxide dismutase activity was unaffected in treated and untreated ischemic rats (Fig. 2E). *B. monniera* gradually improved the glutathione activity though the GPx activity was not significantly affected in ischemic rat (Fig. 2F).

4. Discussion

B. monniera (Brahmi) is a popular drug prescribed by the Indian alternative medicine system practitioners in India since time immemorial. The exact mechanism of action of *B. monniera* could be attributed to a combination of cholinergic modulation (Das et al., 2002; Kishore and Singh, 2005) and antioxidant effects (Bhattacharya et al., 2000a). In the absence of tangible results with the single molecule approach, herbal extracts comprising several molecules are being tested for neuroprotective and therapeutic effects. The neuroprotective effect of three oral doses of *B. monniera* namely, 120 mg kg^{-1}, 160 mg kg^{-1} and 240 mg kg^{-1} was tested in ischemic rats including sodium selenite as reference. In this study, we report that all doses of *B. monniera* (120 mg kg^{-1}, 160 mg kg^{-1} and 240 mg kg^{-1} orally) reversed the ischemia induced memory impairment in the plus maze and passive avoidance tasks better than that of sodium selenite. This finding is supported by our previous studies where *B. monniera* reversed L-NNA (Saraf et al., 2009), scopolamine

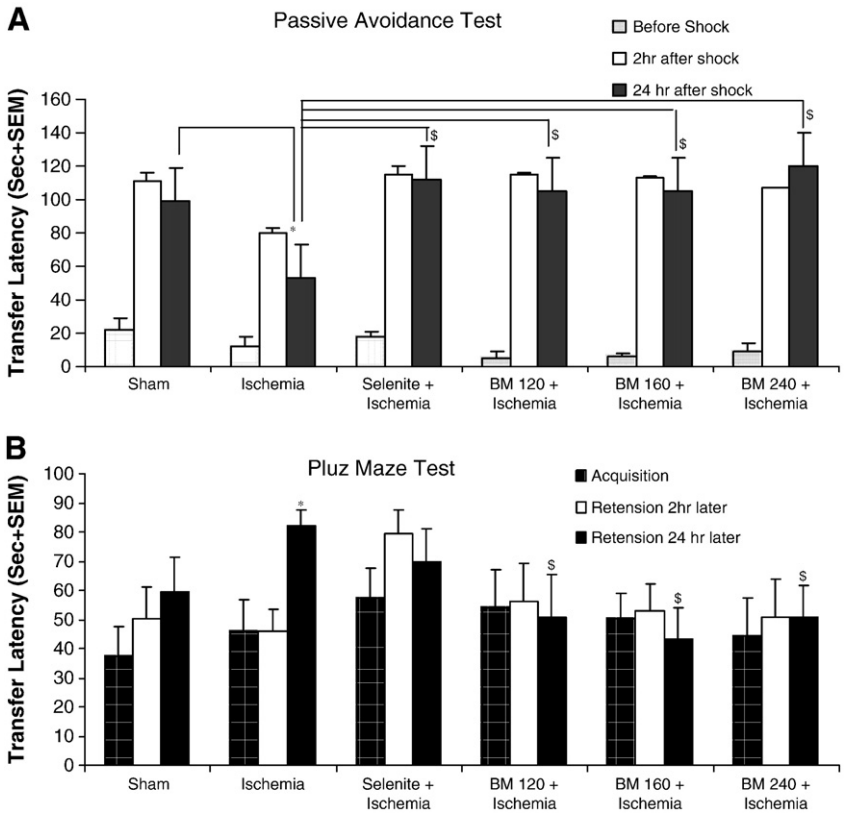

Fig. 1. *B. monniera* attenuates ischemia induced memory deficit. (A) *B. monniera* (120 mg kg^{-1}, 160 mg kg^{-1} and 240 mg kg^{-1} orally) and sodium selenite significantly reverse the reduction in transfer latency as compared to ischemic rats noted after 24 h of delivery of electric shock in passive avoidance test. Transfer latency was not significantly different in treated rat and ischemic rats as compared to control rat before and 2 h after delivery of foot shock. (B) *B. monniera* (120 mg kg^{-1}, 160 mg kg^{-1} and 240 mg kg^{-1} orally), but not sodium selenite, significantly attenuates the impairment in retention as compared to ischemic rat measured 24 h later in the plus maze test. The acquisition and retention 2 h later were unaffected in ischemic and treated rats as compared to control rats. Data were analyzed by ANOVA followed by post hoc 'least significant difference' (LSD). '*' indicates a significant difference for treated group vs control group at $p<0.05$. '$' indicates a significant difference for treated group vs ischemic group at $p<0.05$.

(Saraf et al., 2007) and diazepam induced acquisition and retrieval of memory (Prabhakar et al., 2008). It is reported to improve the performance of rats in various learning situations (Singh and Dhawan, 1982). *B. monniera* extract also reverses Y-maze performance and open field hyperlocomotion behavioral changes and reduces the level of amyloid especially Abeta 1-40 and 1-42 (Holcomb et al., 2006). It provides protection from phenytoin (an antiepileptic drug) induced deficit in cognitive function of mice by similar behavioral tasks (Vohora et al., 2000) lending versatility to its mechanism of action.

In this study, *B. monniera* reduced the infarct size similar to sodium selenite thereby validating the neuroprotective effects of this drug. Moreover, we also found improvement in the neurological scores such as neurodeficit score and fore limb muscle grip strength when *B. monniera* was administered. These observations clearly suggest a protective effect of *B. monniera.* In the previous study *B. monniera* has been documented to provide neuroprotection against cigarette smoke induced apoptosis (Anbarasi et al., 2005a,b,c, 2006a,b) and aluminium induced oxidative stress (Jyoti et al., 2007). Several other herbal drugs have also shown the promising neuroprotective effect in rodents (Chen et al., 2003; Kim et al., 2008; Lee et al., 2003; Sun et al., 2008; Tian et al., 2008; Zhou et al., 2009). Some of those have shown both antiamnesic effects and neuroprotective effects (Kim et al., 2008; Lin et al., 2005; Siripurapu et al., 2005; Wang et al., 2005; Wang et al., 2008; Yun et al., 2007).

The biochemical investigations of the current study have revealed that *B. monniera* reduces the total nitrite and lipid peroxidation, which suggests that *B. monniera* may reduce the formation of a free radical. We also noticed that it enhanced catalase enzyme activity along with mild improvement in glutathione peroxidase activity, although such improvement in glutathione peroxidase activity was not significant. On the other hand, Chowdhuri et al. (2002) reported that the bacoside of *B. monniera* (BBM) attenuates the stress induced suppression of hsp70 expression only in the hippocampus and the cerebral cortex while the cerebellum and the rest of the brain remain unaffected. Moreover the alteration in SOD activity is not homogenous in the

Table 1
B. monniera improves the neurobehavioral outcome in ischemic rats. Neurobehavioral tests such as neurodeficit scoring, pole fall and hanging wire results in impairment of neurobehavioral deficit in ischemic rat. Some of the neurobehavioral outcomes (such as neurodeficit score and fore limb muscle grip strength) showed moderate improvement by *B. monniera* treatment while muscle grip strength measured by pole fall remained unaffected in *B. monniera* treated group.

Group no.	Group name	Neurodeficit mean score	Hanging wire (mean fall time in seconds)	Pole fall (mean fall time in seconds)	Rota rod (mean fall time in seconds)
1	Sham (control)	0	84.23 ± 14.66	31.38 ± 12.6	2.74 ± 0.12
2	Ischemia	3	44.57 ± 18.29	14.92 ± 10.2	1.9 ± 0.047
3	Selenite + ischemia	1	60.78 ± 4.37	31.43 ± 7.46	2.0 ± 0.072
4	BM 120 + ischemia	1.48	78.12 ± 15.62	20.12 ± 9.77	1.38 ± 0.042
5	BM 160 + ischemia	1.71	78.78 ± 9.3	21.85 ± 10.3	1.78 ± 0.069
6	BM 240 + ischemia	1.25	51.23 ± 16.7	21.4 ± 14.2	2.0 ± 0.039

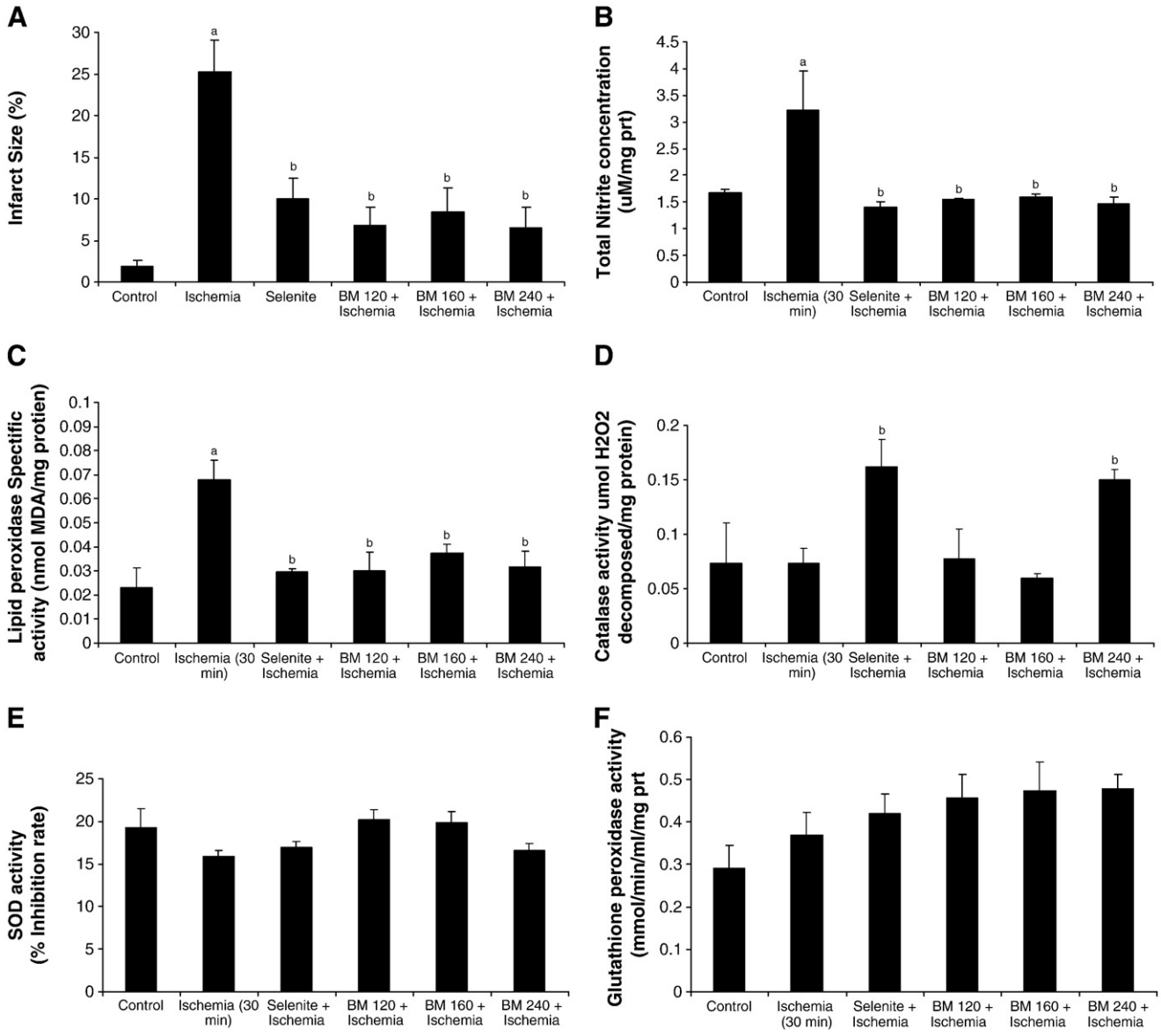

Fig. 2. *B. monniera* attenuates ischemia induced infarction by suppressing molecules involved in oxidative stress. (A) Histogram of size of infarction in control and treated ischemic rats. (B) *B. monniera* and sodium selenite reduced the enhanced total nitrite in ischemic rat as compared to untreated ischemic rat. (C) Similar to sodium selenium, *B. monniera* also reduced the lipid peroxidation in ischemic rat as compared to non-treated ischemic rat. (D) The catalase activity was significantly enhanced in selenite and *B. monniera* (240 mg kg^{-1} orally) treated rats while the lower dose of *B. monniera* (120 mg kg^{-1} and 160 mg kg^{-1} orally) did not affect the catalase activity as compared to control. (E) The superoxide dismutase activity was unaffected in treated and untreated ischemic rats. (F) *B. monniera* gradually improved the glutathione activity as compared to control, though the GPx activity was not significantly affected in ischemic rat. Data were analyzed by ANOVA followed by a least significant difference (LSD). 'a' indicates significant difference for treated group vs control group at $p<0.05$. 'b' indicates a significant difference for treated group vs ischemic group at $p<0.05$.

hippocampus, cortex, cerebellum and the rest of the brain. In this study, we measured the SOD activity in the whole brain rather than any specific part of the brain which explains, in some extent, the absence of change of SOD activity. Chowdhuri et al. (2002) also argued that BBM and stress, when imparted alone, increase the cytochrome P450 but when BBM is administered to the stressed animal it attenuates cytochrome P450 (Chowdhuri et al., 2002). These observations suggest that *B. monniera* may be involved in potentiating the antioxidative defence mechanisms by involving lipid peroxidase and catalase enzymes. This postulation is evidenced by several reports in which *B. monniera* attenuates the cigarette smoke induced neural injury and the cognitive deficit possibly by exhibiting free radical scavenging and anti-lipid peroxidative effects that protect the brain from oxidative damage. It is also known to augment vitamin C, vitamin E, and vitamin A and the activities of glutathione, superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase (Anbarasi et al., 2006b). These investigations establish many leads for future investigations which may include P450, ROS and calcium estimations. Metal ions such as aluminium (Deloncle et al., 2002, 1990, 1995Deloncle and Guillard, 1990), zinc (Choi, 1996; Cuajungco and Lees, 1996; Kim et al., 1999; Koh and Choi, 1994), lead (Han et al., 2007; Nowak et al., 2008) and pollutant (Anbarasi et al., 2006a,c) are contributing factors of the oxidative mechanism that are potential targets which can be screened for mediation by *B. monniera.*

5. Conclusion

On the basis of our findings, we conclude that *B. monniera* attenuates the ischemia induced memory and other neurological deficits including infarct size by exerting antioxidant effects, however, additional investigation on P450, ROS, calcium etc. can uncover the mechanism. Further investigations with purified active constituents of

B. monniera may also validate its neuroprotective and antiamnesic effects. It also suggests that the pursuit of reductionist or single molecule approach should not be a paradigm barrier in clinical translation particularly if herbal extracts promise therapeutic solutions.

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References

Anbarasi K, Vani G, Balakrishna K, Devi CS. Creatine kinase isoenzyme patterns upon chronic exposure to cigarette smoke: protective effect of bacoside A. Vascul Pharmacol 2005a;42:57–61.
Anbarasi K, Vani G, Balakrishna K, Devi CS. Effect of bacoside A on membrane-bound ATPases in the brain of rats exposed to cigarette smoke. J Biochem Mol Toxicol 2005b;19:59–65.
Anbarasi K, Vani G, Devi CS. Protective effect of bacoside A on cigarette smoking-induced brain mitochondrial dysfunction in rats. J Environ Pathol Toxicol Oncol 2005c;24:225–34.
Anbarasi K, Kathirvel G, Vani G, Jayaraman G, Shyamala Devi CS. Cigarette smoking induces heat shock protein 70 kDa expression and apoptosis in rat brain: modulation by bacoside A. Neuroscience 2006a;138:1127–35.
Anbarasi K, Vani G, Balakrishna K, Devi CS. Effect of bacoside A on brain antioxidant status in cigarette smoke exposed rats. Life Sci 2006b;78:1378–84.
Bederson JB, Pitts LH, Tsuji M, Nishimura MC, Davis RL, Bartkowski H. Rat middle cerebral artery occlusion: evaluation of the model and development of a neurologic examination. Stroke 1986;17:472–6.
Bhattacharya SK, Bhattacharya A, Kumar A, Ghosal S. Antioxidant activity of *Bacopa monniera* in rat frontal cortex, striatum and hippocampus. Phytother Res 2000a;14:174–9.
Bhattacharya SK, Kumar A, Ghosal S. Effect of *Bacopa monniera* on animal models of Alzheimer's disease and perturbed central cholinergic markers of cognition in rats. In: Siva Sanka DV, editor. Molecular aspects of Asian medicines. New York: PJD Publication; 2000b.
Chatterji 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.
Chen M, Wang Y, Liu Y, Hou XY, Zhang QG, Meng FJ, et al. Possible mechanisms underlying the protective effects of SY-21, an extract of a traditional Chinese herb, on transient brain ischemia/reperfusion-induced neuronal death in rat hippocampus. Brain Res 2003;989:180–6.
Choi DW. Zinc neurotoxicity may contribute to selective neuronal death following transient global cerebral ischemia. Cold Spring Harb Symp Quant Biol 1996;61:385–7.
Chowdhuri DK, Parmar D, Kakkar P, Shukla R, Seth PK, Srimal RC. Antistress effects of bacosides of *Bacopa monnieri:* modulation of Hsp70 expression, superoxide dismutase and cytochrome P450 activity in rat brain. Phytother Res 2002;16:639–45.
Cuajungco MP, Lees GJ. Prevention of zinc neurotoxicity in vivo by N, N, N′, N′-tetrakis (2-pyridylmethyl) ethylene-diamine (TPEN). NeuroReport 1996;7:1301–4.
Das A, Shanker G, Nath C, Pal R, Singh S, Singh H. A comparative study in rodents of standardized extracts of *Bacopa monniera* and *Ginkgo biloba*: anticholinesterase and cognitive enhancing activities. Pharmacol Biochem Behav 2002;73:893–900.
del Zoppo GJ, Hallenbeck JM. Advances in the vascular pathophysiology of ischemic stroke. Thromb Res 2000;98:73–81.
Delaney CA, Green IC, Lowe JE, Cunningham JM, Butler AR, Renton L, et al. Use of the comet assay to investigate possible interactions of nitric oxide and reactive oxygen species in the induction of DNA damage and inhibition of function in an insulin-secreting cell line. Mutat Res 1997;375:137–46.
Deloncle R, Guillard O. Mechanism of Alzheimer's disease: arguments for a neurotransmitter-aluminium complex implication. Neurochem Res 1990;15:1239–45.
Deloncle R, Guillard O, Clanet F, Courtois P, Piriou A. Aluminum transfer as glutamate complex through blood-brain barrier. Possible implication in dialysis encephalopathy. Biol Trace Elem Res 1990;25:39–45.
Deloncle R, Guillard O, Huguet F, Clanet F. Modification of the blood-brain barrier through chronic intoxication by aluminum glutamate. Possible role in the etiology of Alzheimer's disease. Biol Trace Elem Res 1995;47:227–33.
Deloncle R, Fauconneau B, Piriou A, Huguet F, Guillard O. Aluminum L-glutamate complex in rat brain cortex: in vivo prevention of aluminum deposit by magnesium D-aspartate. Brain Res 2002;946:247–52.
Dhawan BN, Singh HK. Nootropic and some other effects of *Bacopa monniera* Linn. (Brahmi). Ann Neurosci 2002;6:34–40.
Epe B, Ballmaier D, Roussyn I, Briviba K, Sies H. DNA damage by peroxynitrite characterized with DNA repair enzymes. Nucleic Acids Res 1996;24:4105–10.
Fiskum G. Mitochondrial participation in ischemic and traumatic neural cell death. J Neurotrauma 2000;17:843–55.
Han JM, Chang BJ, Li TZ, Choe NH, Quan FS, Jang BJ, et al. Protective effects of ascorbic acid against lead-induced apoptotic neurodegeneration in the developing rat hippocampus in vivo. Brain Res 2007;1185:68–74.
Holcomb LA, Dhanasekaran M, Hitt AR, Young KA, Riggs M, Manyam BV. *Bacopa monniera* extract reduces amyloid levels in PSAPP mice. J Alzheimers Dis 2006;9:243–51.
Hou ST, MacManus JP. Molecular mechanisms of cerebral ischemia-induced neuronal death. Int Rev Cytol 2002;221:93-148.
Jyoti A, Sethi P, Sharma D. *Bacopa monniera* prevents from aluminium neurotoxicity in the cerebral cortex of rat brain. J Ethnopharmacol 2007;111:56–62.
Kim EY, Koh JY, Kim YH, Sohn S, Joe E, Gwag BJ. Zn2+ entry produces oxidative neuronal necrosis in cortical cell cultures. Eur J Neurosci 1999;11:327–34.
Kim YT, Yi YJ, Kim MY, Bu Y, Jin ZH, Choi H, et al. Neuroprotection and enhancement of spatial memory by herbal mixture HT008-1 in rat global brain ischemia model. Am J Chin Med 2008;36:287–99.
Kishore K, Singh M. Effect of bacosides, alcoholic extract of *Bacopa monniera* Linn. (Brahmi), on experimental amnesia in mice. Indian J Exp Biol 2005;43:640–5.
Koh JY, Choi DW. Zinc toxicity on cultured cortical neurons: involvement of N-methyl-D-aspartate receptors. Neuroscience 1994;60:1049–57.
Kumar MH, Gupta YK. Antioxidant property of *Celastrus paniculatus* willd.: a possible mechanism in enhancing cognition. Phytomedicine 2002;9:302–11.
Lee B, Choi Y, Kim H, Kim SY, Hahm DH, Lee HJ, et al. Protective effects of methanol extract of Acori graminei rhizoma and Uncariae ramulus et Uncus on ischemia-induced neuronal death and cognitive impairments in the rat. Life Sci 2003;74:435–50.
Lin Z, Yan Y, Zhu D, Yu B, Wang Q. Protective effects of FBD—an experimental Chinese traditional medicinal formula on memory dysfunction in mice induced by cerebral ischemia–reperfusion. J Ethnopharmacol 2005;97:477–83.
Luck H. Catalase. In: Bergmeyer HO, editor. Methods of Enzymatic analysis. New York, London: Academic Press; 1971. p. 885–93.
Moskowitz N, Schook W, Puszkin S. Regulation of endogenous calcium-dependent synaptic membrane phospholipase A2. Brain Res 1984;290:273–9.
Nowak P, Szczerbak G, Nitka D, Kostrzewa RM, Josko J, Brus R. Cortical dopaminergic neurotransmission in rats intoxicated with lead during pregnancy. Nitric oxide and hydroxyl radicals formation involvement. Neurotoxicol Teratol 2008;30(5):428–32.
O'Brien JT, Erkinjuntti T, Reisberg B, Roman G, Sawada T, Pantoni L, et al. Vascular cognitive impairment. Lancet Neurol 2003;2:89–98.
Ollinger K, Brunk UT. Cellular injury induced by oxidative stress is mediated through lysosomal damage. Free Radic Biol Med 1995;19:565–74.
Prabhakar S, Saraf MK, Pandhi P, Anand A. *Bacopa monniera* exerts antiamnesic effect on diazepam-induced anterograde amnesia in mice. Psychopharmacology (Berl) 2008;200:27–37.
Rensink M, Schuurmans M, Lindeman E, Hafsteinsdottir T. Task-oriented training in rehabilitation after stroke: systematic review. J Adv Nurs 2009;65(4):737–54.
Russo A, Izzo AA, Borrelli F, Renis M, Vanella A. Free radical scavenging capacity and protective effect of *Bacopa monniera* L. on DNA damage. Phytother Res 2003;17:870–5.
Sacco RL, Adams R, Albers G, Alberts MJ, Benavente O, Furie K, et al. Guidelines for prevention of stroke in patients with ischemic stroke or transient ischemic attack: a statement for healthcare professionals from the American Heart Association/American Stroke Association Council on Stroke: co-sponsored by the Council on Cardiovascular Radiology and Intervention: the American Academy of Neurology affirms the value of this guideline. Stroke 2006;37:577–617.
Saraf MK, Prabhakar S, Khanduja KL, Anand A. Antiamnesic action of *Bacopa monniera* is mediated through protein kinase-CREB. Pathway in amnesic mice induced by Scopolamine. San Diego, CA, USA: Society for Neuroscience; 2007. p. 156. 19/U3.
Saraf MK, Prabhakar S, Anand A. *Bacopa monniera* alleviates N-nitro-L-arginine-induced but not MK-801-induced amnesia: a mouse Morris water maze study. Neuroscience 2009;160(1):149–55.
Shanker G, Singh HK. Anxiolytic profile of standardized Brahmi extract. Ind J Phamacol 2000;32:152.
Singh HK, Dhawan BN. Effect of *Bacopa monniera* Linn. (Brahmi) extract on avoidance responses in rat. J Ethnopharmacol 1982;5:205–14.
Singh HK, Dhawan BN. Neuropsychopharmacological effects of the ayurvedic nootropic *Bacopa monniera* Linn. (Brahmi). Ind J Pharmacol 1997;29S:359–65.
Singh RH, Singh L. Studies on the antioxidant anxiety effect of the Medhay Rasayan drug Brahmi (*Bacopa monniera* Linn)—Part II (experimental studies). J Res Ind Med. Yoga Homeo 1980;14:1–6.
Singh HK, Rastogi RP, Srimal RC, Dhawan BN. Effects of bacosides A and B on avoidance response in rats. Phytother Res 1988;2:70–5.
Siripurapu KB, Gupta P, Bhatia G, Maurya R, Nath C, Palit G. Adaptogenic and anti-amnesic properties of *Evolvulus alsinoides* in rodents. Pharmacol Biochem Behav 2005;81:424–32.
Sun YF, Pei DS, Zhang QX, Zhang GY. Neuroprotection of GST, an extract of traditional Chinese herb, against ischemic brain injury induced by transient brain ischemia and reperfusion in rat hippocampus. Neurol Res 2008;30:471–5.
Tian J, Li G, Liu Z, Zhang S, Qu G, Jiang W, et al. ND-309, a novel compound, ameliorates cerebral infarction in rats by antioxidant action. Neurosci Lett 2008;442:279–83.
Tripathi YB, Chaurasia S, Tripathi E, Upadhyay A, Dubey GP. *Bacopa monniera* Linn. as an antioxidant: mechanism of action. Indian J Exp Biol 1996;34:523–6.
Vohora D, Pal SN, Pillai KK. Protection from phenytoin-induced cognitive deficit by *Bacopa monniera*, a reputed Indian nootropic plant. J Ethnopharmacol 2000;71:383–90.
Wang Z, Li YM, Gong XJ, Lin J, Zou JM. Effects of Naomaitai capsule on learning and memory and content of Ach in rat model produced by cerebral ischemia–reperfusion. Zhongguo Zhong Yao Za Zhi 2005;30:459–62.
Wang ZF, Yan J, Fu Y, Tang XC, Feng S, He XC, et al. Pharmacodynamic study of FS-0311: a novel highly potent, selective acetylcholinesterase inhibitor. Cell Mol Neurobiol 2008;28:245–61.
Wills ED. Mechanisms of lipid peroxide formation in animal tissues. Biochem J 1966;99(3):667–76.
Yun YJ, Lee B, Hahm DH, Kang SK, Han SM, Lee HJ, et al. Neuroprotective effect of palmul-chongmyeong-tang on ischemia-induced learning and memory deficits in the rat. Biol Pharm Bull 2007;30:337–42.
Zhou Y, Peng L, Zhang WD, Kong DY. Effect of triterpenoid saponins from *Bacopa monniera* on scopolamine-induced memory impairment in mice. Planta Med 2009;75:568–74.