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Experimental and Clinical Evaluation of Nootropic Activity of Bacopa monniera Linn. (Brahmi)

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SUMMARY

Bacopa monniera Linn. (Brahmi) is an annual creeper belonging to family Scrophulariaceae and growing all over the Indian sub-continent in marshy areas. It is a major *Medhya Rasayana* used in *Ayurveda* for treatment of memory disorders. Large number of saponins and glycosides has been isolated from the plant. Most of the experimental and clinical studies have been done with crude extracts or standardized preparation of the two active saponins Bacosides A and B.

Extracts or saponin mixture facilitate learning, improve consolidation of learned behavior and delay extinction in several models of learnt behavior in normal rats and mice as well as in chemically induced or transgenic models of Alzheimer's disease. They also prevent or reverse amnesia produced by drugs, stress or ischemic hypoxia. Other CNS effects include anti-anxiety, anti-convulsant and analgesic activity. Several mechanisms have been proposed to explain the mechanism of these CNS effects.

Extracts as well as the bacoside preparation have been found safe and well tolerated in healthy volunteers in single dose or chronic administration for several weeks in a number of double blind placebo controlled studies in India and abroad. Chronic administration significantly improved information processing, learning and memory consolidation. It was found more effective than caffeine in a comparative study.

Double blind placebo controlled studies with bacoside preparation have demonstrated beneficial effects and safety in elderly patients with Age Related Memory Impairment and in children with Attention Deficit Memory Disorder. It has also been found useful in anxiety neurosis, epilepsy and sleep disturbances in post menopausal women.

The standardized preparation is marketed as a prescription drug after having obtained the necessary regulatory approval in India, Australia, New Zealand and South Africa and as an OTC product in several other south east Asian and African countries.

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Introduction

Bacopa monniera Linn. (Syn. *Herpestis monniera* Linn H.B. & K, *Brahmi)* is an annual creeper belonging to family Scrophulariaceae and found all over the Indian sub-continent in damp and marshy areas. It is an important plant in *Ayurvedic* materia medica. It is classified in *Charak Samhita* as a *Medhya rasayana* for improvement of memory and described in *Sushruta Samhita* as being efficacious in loss of intellect and memory(1). It has been as a single herb and also in formulations with other ingredients. *Centella asiatica* (*Hydrocotyle asiatica)* is also used in Ayurveda for similar indications and thee is often confusion between these 2 plants. Singh and Sinha (2) have clarified that *Bacopa* is *Brahmi* and *Centella* is *mandookparni.* They have also stated that the former is more potent and used as drug while the later is recommended as a dietary constituent (*Saka dravya* or vegetable).

Chemical studies on the plant were initiated in 1931 by Bose and Bose (3) with isolation of the alkaloid Brahmine followed by isolation of a saponin, hersaponin by Sastri *et al* in 1969 (4). Detailed chemical analysis was undertaken at Central Drug Research Institute Lucknow (CDRI), leading to isolation of the major saponins, Bacosides A and B (5-7). Bacoside A was subsequently shown to be a mixture of 4 aglycones, Bacogenins A_{1-4} (8-10). Other minor constituents isolated at CDRI were Bacoside A_1 (11) and A_3 (12). Several minor constituents have been isolated subsequently by investigators elsewhere. These include: 4 dammarane type triterpinoid Bacosaponins A-D (13, 14); 2 pseudojujubogenin glycosides Bacoposides I and II (15) and saponins Bacopasides III-V (16) and Bacosaponin G (17). Most of the experimental studies have been done with crude extracts or standardized mixture of Bacosides A and B (Bacoside mixture) developed at CDRI

Experimental Studies

Major emphasis in experimental studies has been on analyzing its effect on learning and memory but some other CNS effects have also been reported. The major findings have been summarized below under effects on learning and memory, anti-amnesic activity and other CNS effects.

Effects on Learning and Memory

Prakash and Sirsi (18) published the first report in 1962 on improvement of performance of rats in motor learning with alcoholic extract. Sinha (19) reported facilitation of acquisition, consolidation and retention with the glycoside, hersaponin, in a brightness discrimination test. Improvement in maze learning by rats with a decoction was observed by Dey *et al* (20).

A more detailed study has been at CDRI, initially with the alcoholic extract in rats (21). Animals treated with 40 mg/kg extract per oral for 3 or more days showed better acquisition, improved

retention and delayed extinction in a shock motivated brightness discrimination test. It also reduced reaction time significantly in an active conditioned flight test and improved performance in Sidman's continuous response test. Similar effect was obtained in the first 2 tests with a much lower dose (2.5-7.5 mg/kg) of Bacoside mixture. It also significantly reduced lithium chloride intake in conditioned taste aversion test (22). It abolished the 'Kamin's deficit' (23) in the re-learning schedule of Y-maze test (24). Improvement in learning has been confirmed in rat (25) and mouse (26, 27) from other laboratories.

Bhattacharya *et al* (28) and Uabundit *et al* (29) have shown its beneficial effect in a rat model of Alzheimer disease. Rastogi *et al* (30) have found that long term (3 months) treatment with bacosides prevented age-associated neuronal degeneration in female Wistar rats. Charles *et al* (31) have reported similar reversal of galactose induced attenuation of contextual associated learning in ageing rats. A reduction in β-amyloid level in brain associated with improvement in Y-maze performance and open field hyper-locomotion has been obtained in doubly transgenic PSAPP mice (32). Protection against β-amyloid induced cell death has been observed in primary cortical cell culture also (33). These results are suggestive of its potential utility in patients of Alzheimer's disease.

Anti-amnesic Activity

Bacosides reverse retrograde amnesia in rats produced by immobilization stress, electroconvulsive shock or scopolamine (1).Reversal of scopolamine amnesia has also been reported by Manjarekar (34) and Das *et al* (35). Studies in mice have shown their ability to reverse amnesia induced by diazepam (36), NOS inhibitor L-NNA (37), phenytoin (38), 1-(m-chlorophenyl) biguanide (39) hypobaric hypoxia (40) and ischemia (41).

Other CNS Effects

Extracts and pure compounds isolated from *Bacopa* have been shown to have tranquilizing (42), anti-anxiety (43-45), anti-depressant (45-47), anti-convulsant (48) and analgesic (49) activities. Antagonism of haloperidol induced catalepsy also has been reported (50).

Studies on Mechanism of Action

Bacosides enhanced protein kinase activity in hippocampus, hypothalamus and cerebral cortex (1). They also prevent decrease in SOD, intra-neuronal lipofuschin accumulation and necrotic changes induced by aluminum trichlorate in CA-1 region of hippocampus (51) and cerebral cortex (52). Bhattacharya *et al* (53) have reported anti-oxidant, free radical scavenging and anti-lipid peroxidation effect of *Bacopa* extract. Rasso *et al* (54) have found protection against NOS inhibition

evidenced by altered NO synthesis, reduction in intracellular oxidants and prevention of DNA damage in cultured astrocytes. The studies of Saraf *et al* (36) in mice suggest that anti-amnesic activity may be partly due to restoration of NO release by reducing NOS inhibition. The protection from oxidative damage is achieved by maintaining functional integrity of mitochondria (55) and membrane ionic balance (56). Dhanasekeran *et al* (57) observed reduced concentration of divalent metals in addition to reduction in lipid peroxides and lipoxygenase activity. They suggest a role of the metals in reduction of β-amyloid in brain of Alzheimer's disease animal models.

Kar Choudhury *et al* (58) have conducted studies in stressed rats. The decrease in Hsp_{70} expression and SOD release was blocked. Similar results have been obtained by Annbarasi *et al* (59) in animals exposed to cigarette smoke.

Several neurotransmitters may be involved in nootropic activity of *Bacopa* preparations. An increase in 5-HT and lowering of norepinephrine has been found in hippocampus, hypothalamus and cerebral cortex of adult rats treated with bacosides without any effect on their receptors (1). Charles *et al* (60) treated young rats with *Bacopa* extract on post-natal days 15-29. Their results suggest that nootropic activity may be mediated through regulation of expression of tryptophan hydroxylase (TPH_2) leading to raised 5-HT level. Dopamine levels decreased significantly but no changes were obtained in levels of glutamate or acetylcholine. Das *et al* (35), however, found inhibition of acetyl cholinesterase in mice brain and suggested involvement of a cholinergic mechanism. Limpeanchob *et al* (33) also found a reduction of β-amyloid induced increase in acetyl cholinesterase activity in cultured cortical neurons with a *Bacopa* extract. Bacoside-A pre-treatment could revert fall in GABA receptors in hippocampus of rat model of temporal epilepsy (61). The authors suggest possibility of modulation by a cholinergic mechanism

Kamkaew *et al* (62) studied the effect of alcoholic extract of *Bacopa monniera* on cerebra blood flow in rats. There was 25% increase in cerebral blood flow without any effect on systemic blood pressure. They suggest a role of improved blood supply in the nootropic effect.

Preethi *et al* (63) have demonstrated down-regulation of micro RNA-24 by *Bacopa* extract in young rats. It has been postulated that this would result in up-regulation of CREB which regulates activation of immediate early genes facilitating synaptic plasticity (64). *p*.$CREB_1$ is involved in regulation of synthesis of synaptic proteins necessary for consolidation of long term memory (65).

Clinical Studies

Normal Volunteers

The first Phase I study under GCP norms was undertaken at CDRI with standardized Bacoside preparation (CDRI

formulation) after generating the required pre-clinical efficacy and safety (acute and chronic toxicity, teratogenicity and mutagenicity) data and obtaining approval from the Drugs' Controller General of India. The double blind placebo controlled study was conducted in male volunteers after obtaining informed consent. It was well tolerated and devoid of untoward effect in single (200-300mg) or multiple (100 and 200 mg daily for 4 weeks) dose schedules (1, 66). Pravina *et al* (67) found similar results in an open study in 23 volunteers given 300mg daily for 15 days followed by 450 mg for next 15 days.

Nathan *et al* (68) studied effect of single 300 mg dose of CDRI formulation in a placebo controlled double blind study in 38 volunteers. It was found safe but had no effect on cognitive functioning. Administration of same dose for 3 months led to significant improvement in information processing, learning and memory consolidation judged by storage and retention of new information (69, 70).

Mandal *et al* (71) gave 750 mg whole plant powder daily for 16 weeks in a placebo controlled double blind study. Significant facilitation was observed in verbal span test, verbal memory task and text comprehension tests. Raina *et al* (72) compared the effect of 500 mg plant powder with 200 mg caffeine daily for 16 weeks in 40 volunteers. *Bacopa* powder was better than caffeine in improving reaction time in a battery of cognitive tests with fewer side effects.

Senior Citizens with Memory Impairment

Most of the studies have been done with CDRI formulation. Raghav *et al* (73) evaluated the effect of 12 weeks treatment in 40 subjects having Age-associated Memory Impairment without any evidence of dementia or psychiatric disorder. The study was double blind randomized. It significantly improved mental control, logical memory and paired associated learning without any drug related abnormality in clinical, hematological or biochemical parameters. Significant improvement in logical memory, digit forward, paired associated learning and total score was obtained in a study at another centre also (74). Similar results regarding efficacy and safety has been obtained in several other placebo controlled double blind studies in India (75, 76) and abroad (77, 78).

Morgan and Stevens (77) observed more gastrointestinal side effects than in the placebo treated group and suggest that these may be due to its cholinergic effects. Agrawal (79) has reported that treatment with *Brahmi* powder prevented depletion of blood acetylcholine in patients of psychosomatic disorders.

Children with Impaired Learning

A double blind placebo controlled study has been done with CDRI formulation in 40 children with Attention Deficit Memory Disorder (80). The drug or placebo was given for 12 weeks. Significant improvement was observed in

tests for mental control, sentence repetition, logical memory, word or picture recall and paired associated learning. Sharma *et al* (81) reported improvement in perpetual motor function in a placebo controlled trial in 40 school going children. Abhang (82) carried out a double blind study for one month in 100 male students (10-13 years age) with subnormal IQ. There was improvement in direct memory, some verbal factors and arithmetic skill.

Other CNS Disorders

Mukherjee and Day (83) published the first clinical with Brahmi in 1966. They compared the effect of defatted alcoholic extract (2-4 mg/kg) with aqueous extract (2 oz/day) for 5 months in patients of epilepsy and found the former more effective. In a follow up study Dey (84) showed a close parallelism in clinical improvement and EEG changes in 2 of these patients

Singh and Singh (85) treated 30 cases of anxiety neurosis with 30 ml extract (prepared from 12g of crude drug) in two divided doses for one month. There was significant relief in symptoms associated with a reduction in urinary excretion of vinyl mandellic acid and corticoids. Subsequently they gave dried extract of 2.5g crude drug in capsule thrice daily for 4 weeks to 18 normal subjects and same number of patients of anxiety neurosis (86). There was significant improvement in symptoms of anxiety and depression, mental fatigue and memory span.

Kumar *et al* (87) found significant improvement in anxiety and stress level with the CDRI formulation given to 94 adult volunteers for 6 months in a double blind placebo controlled cross-over study. Other recent studies have reported improvement in quantity and quality of sleep in post-menopausal women (88) and in the range of movement and joint pain etc. in patients of sciatica (89).

Concluding Remarks

The CDRI formulation has been available as a prescription drug for memory disorders in India since 1994 under several trade names. It has been subsequently being marketed as a prescription drug for same indications in Australia, New Zealand since 2009 and South Africa since 2011. It was made available as an OTC product in Sri Lanka in 1996 followed by Philippines (largest selling natural product), Malaysia (among top 5 selling herbal drugs), Singapore, Thailand and several African countries.

Bacopa preparations show a wide spectrum of CNS effects in experimental studies. Initial clinical studies (reviewed above) indicate beneficial effect in anxiety neurosis and epilepsy. This data along with animal and clinical safety data suggest the need of more extensive clinical trials in these and other relevant CNS disorders to assess its potential as primary treatment or adjunct to other drugs. They may also be useful in ameliorating detrimental effects of drugs like benzodiazepines or anticonvulsant drugs on cognitive function in patients

using these drugs for long periods. Experimental studies also suggest their potential utility in management of stressful conditions (90) and morphine abstinence syndrome (91).

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