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Bacopa monniera leaf extract up-regulates tryptophan hydroxylase (TPH2) and serotonin transporter (SERT) expression: Implications in memory formation

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ABSTRACT

Aim of the study: To examine the effect of *Bacopa monniera* leaf ethanolic extract (BMEE) on the serotonergic system of postnatal rats with reference to learning and memory.
Materials and methods: From postnatal day (PND)-15–29, rats were treated with BMEE (40 mg/kg BW + 0.5% gum acacia) by oral gavage. Behavioural tests (Y-maze, hole-board and passive avoidance) were used to evaluate their learning (PND-32–37) and retention of memory (PND-47–53). Effect of BMEE on neurotransmitter system was analyzed by ELISA and semi-quantitative polymerase chain reaction (PCR).
Results: Oral administration of BMEE improved learning and retention of memory significantly in all behavioural tasks. Following BMEE treatment, the level of serotonin (5-HT) increased while dopamine (DA) decreased significantly. We also found variation in the level of acetylcholine (ACh). However, no significant changes were observed in the level of ACh and glutamate (Glu). The level of 5-HT was significantly elevated up to PND-37 and was then restored to normal level on PND-53. Interestingly, concomitant up-regulation was recorded in the mRNA expression of serotonin synthesizing enzyme tryptophan hydroxylase-2 (TPH2) and serotonin transporter (SERT) on PND-29 and PND-37, which was restored on PND-53.
Conclusions: The results suggest that BMEE treatment significantly enhances the learning and retention of memory in postnatal rats possibly through regulating the expression of TPH2, 5-HT metabolism and transport.

1. Introduction

Neurotransmitters such as dopamine (DA), serotonin [5-hydroxytryptamine (5-HT)] and norepinephrine (NE) are involved in the basic physiological, behavioural and endocrine functions (Greengard, 2001). Studies have correlated the memory performance with the systems extracellular 5-HT level, and depletion of tryptophan could affect the memory formation (van der Veen and Evers, 2006; Karakuyu et al., 2007). Biosynthesis of 5-HT is regulated by the rate-limiting enzyme tryptophan hydroxylase (TPH), the elevation of its mRNA expression has been reported to enhance TPH activity and 5-HT synthesis (Chamas et al., 1999; Kim et al., 2002). 5-HT is synthesized within 5-HT neuronal cell bodies, most of which are immunoreactive for serotonin transporter (SERT) (Fujita et al., 1993). Clearance of synaptic and extra-synaptic 5-HT is the principal function of SERT; altered SERT expression has been implicated in multiple forms of psychological disorders (Zhao et al., 2006). Several studies have tested the *in vivo* efficacy of plant extracts to identify biologically active compounds that could improve the mental function and learning ability (Khalifa, 2001; Rai et al., 2001; Das et al., 2002; Mohandas Rao et al., 2005; Kimani and Nyongesa, 2008).

Bacopa monniera L. (Family: Scrophulariaceae) has been commonly used in Indian traditional system of Ayurvedic medicine for improvement of memory deficit (Russo and Borrelli, 2005). The ethanolic extract of its leaf contains various active alkaloids such as nicotine, brahmine and herpestine, and triterpenoid saponins such as bacoside A and B (Chatterji et al., 1963, 1965; Kulshreshtha and Rastogi, 1973, 1974; Chandel et al., 1977). Later, several other saponin compounds such as bacopaside I, II, III, IV and V were identified (Chakravarty et al., 2001, 2003). Treatment of the leaf extract of *Bacopa monniera* alleviating several aspects of learning and mental function (Singh and Dhawan, 1982, 1997; Vollala et al., 2010), by modulating the levels of monoamine like noradrenaline (NA), DA and 5-HT at different regions of brain (Sheikh et al., 2007). It has also been reported to alter the glutamate receptor binding

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and NMDA R1 gene expression in epileptic rats (Khan et al., 2008), could reduce hypobaric hypoxia induced spatial memory impairment (Hota et al., 2009) and attenuate the N_{ω}-nitro-L-arginine (L-NNA) induced amnesia (Saraf et al., 2009). Furthermore, therapeutic effect of *Bacopa monniera* treatment has been demonstrated to possess neuroprotective effect against the cholinergic degeneration (Uabundit et al., 2010), reversing diazepam (Prabhakar et al., 2008; Saraf et al., 2008) and scopolamine-induced memory deficit (Zhou et al., 2009; Saraf et al., 2010).

Although studies have documented the various pharmacological activities of *Bacopa monniera*, very little is known about its interaction with the serotonergic system. To gain more insight into the interaction of *Bacopa monniera* with serotonergic system during different phases of learning and retention of memory, expression patterns of *TPH2*, *SERT* and the level of 5-HT were studied. Our findings show that BMEE treatment enhances the learning ability and memory, possibly through modulating 5-HT synthesis and its transportation.

2. Materials and methods

2.1. Animals

Postnatal day (PND)-14 Wistar rat pups were housed in rectangular polypropylene cages (43 cm × 27 cm × 15 cm). Paddy husk was used as bedding material which was replaced once in two days. The animals had access to commercial standard rodent chow and fresh water *ad libitum*. The animals were maintained under standard 12:12 h light–dark conditions, constant temperature (22 ± 1 °C), and 60% relative humidity. The experiments were conducted between 10:00 h and 17:30 h in a semi-soundproof laboratory. All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC/BDU/13/2009-10), Bharathidasan University, Tiruchirappalli, India.

2.2. Plant material and preparation of the extract

Bacopa monniera plant was collected from the wild (Tiruchirappalli, 10°48′10.39″N; 78°41′55.40″E), Tamil Nadu, India and was taxonomically identified and authenticated by Rapinat Herbarium, St Josephs College, Tiruchirappalli, India and the specimen is preserved in the herbarium (specimen voucher No. RHT 63872). The shade-dried and powdered leaves of *Bacopa monniera* were weighed and soaked in water for 24 h. Water was discarded and the residual plant material was extracted thrice with ethanol (95%) by maceration (Phrompittayarat et al., 2007). The obtained *Bacopa monniera* ethanolic extract (BMEE) filtrates was pooled and then evaporated to dryness using a rotary evaporator (Buchi Rotavapor, Switzerland) under reduced pressure.

2.3. HPLC analysis of BMEE extract

2.3.1. Sample preparation

The extract 500 mg was dissolved in 50 ml of methanol, sonicated for 10–15 min, cooled, made up to 100 ml with methanol, and filtered through a 0.45 μm membrane filter prior to injection into the chromatographic system.

2.3.2. Instrumentation

According to Deepak et al. (2005), presence of bacoside was determined by using a Shimadzu HPLC system equipped with a SPD-M10 AVP photodiode array detector (PDA). The mobile phase consists of A – 0.25% orthophosphoric acid in water and B – acetonitrile. The analysis took 45 min and the column oven temperature was maintained at 25 °C. The combination of mobile phase A/B at different times were as follows: at 0.00 min 75/25, at 25.00 min 60/40, at 35.00 min 40/60, at 38.00 min 75/25 and 45 min 75/25. The flow rate was 1.5 ml/min and the injection volume was 25.0 μl. Separations were monitored at the wavelength of 205 nm and peak identities were established by comparing the HPLC retention time with the reference compound. Concentration of the bacosides was calculated using the formula described earlier (Muthumary and Sashirekha, 2007; Tiwari et al., 2010). The analysis repeated three times to confirm the presence of compound in the extract. HPLC analysis and calculations were carried out commercially by Natural Remedies Pvt. Ltd., Bangalore, India.

2.4. Dose selection

A pilot study was conducted to establish the optimal dose of BMEE by evaluating behaviour and toxicity. Rat pups aged PND-14 were randomly divided into four groups of eight animals each as follows: (1) control (0.5% gum acacia + double distilled water); (2) experimental group I (20 mg/kg BW + 0.5% gum acacia); (3) experimental group II (30 mg/kg BW + 0.5% gum acacia); experimental group III (40 mg/kg BW + 0.5% gum acacia). During the growth spurt period (PND-15–29) the freshly prepared aqueous suspension was orally administered (10:00–11:00 h) to the rats every day. After the treatment, control and experimental group rats were subjected to Y-maze test to assess their learning ability and retention of memory.

2.5. Assessment of learning and memory

2.5.1. Treatment schedule

PND-14 rat pups were randomly divided into three groups (control, experimental and positive control), each group containing six animals. From PND-15 to 29, the control group rats received 0.5% gum acacia, experimental group rats received BMEE (40 mg/kg BW + 0.5% gum acacia) and positive control group received bacoside A (12.52 mg/kg BW + 0.5% gum acacia).

2.5.2. Behavioural test

Control and experimental group rats were subjected to behavioural tests (Y-maze, hole-board and passive avoidance test) to assess their learning ability and retention of memory. Food restriction maintained as a motivation to animals at 80–85% of their *ad libitum* or control body weight (Toth and Gardiner, 2000). All behavioural tests were conducted by an investigator who was uninformed about the subject's treatment.

2.6. Y-maze test

Rat pups were randomly divided into two groups as follows: (1) control (n = 60); (2) experimental (n = 60). Behavioural testing was conducted in the custom-constructed Y-maze. Control and BMEE treated experimental group rats were allowed to explore the Y-maze for 5 min prior to the training period on PND-30 and 31. Subsequently, the acquisition test was conducted from PND-32 to 37 and retention of memory was tested from PND-46 to 53. The apparatus was constructed with the reported specifications and performance was tested as described previously (Van der Borght et al., 2007). At different phases of behavioural test, set of pups were sacrificed for neurotransmitter and gene expression analysis (details in respective method section).