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Research Article

Effect of *Bacopa monnieri* Extract on Memory and Cognitive Skills in Adult Humans: A Randomized, Double-Blind, Placebo-Controlled Study

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Abstract

Introduction: Age-related cognitive decline affects quality of life and freedom during old age. Early interventions help to maintain normal and healthy cognitive aging. *Bacopa monnieri* is known for its memory-enhancing properties and used widely to improve cognitive functions. This study reports safety and efficacy of *B. monnieri* extract (BME) on memory and cognitive functions in healthy adults. **Methods:** Eighty healthy subjects (40 per group) were evaluated in this randomized, double-blind, placebo-controlled, parallel group study. Subjects consumed either 300 mg of BME containing 90 mg of total bacosides or placebo every morning after breakfast for 12 weeks. Memory (baseline, Days 28, 56 and 84) and cognitive functions (baseline, Days 1, 14, 28, 56 and 84) were measured using Creyos battery of tests; anxiety and sleep quality were assessed by Beck Anxiety Inventory and Pittsburgh Sleep Quality Index, respectively at baseline, Days 28, 56 and 84. Brain Derived Neurotrophic Factor (baseline and Day 84) and cortisol levels (baseline, Days 56 and 84) were analyzed in serum samples. Safety was assessed throughout the study based on physical examination, vital signs, clinical laboratory tests and monitoring of adverse events. **Results:** Thirty-six subjects from placebo group and 38 subjects from BME group completed the study. BME group showed significant improvements on both memory (verbal short-term memory, spatial short-term memory, working memory, visuospatial working memory, and episodic memory) and cognition skills (concentration, alertness, reasoning, and mental flexibility) over placebo from baseline to Day 84, with effects on cognitive skills as early as Day 14 and Day 28 for memory. Further, a significant acute effect on concentration was observed as early as 3 hours post single dose consumption of BME. Anxiety score and sleep quality were significantly improved for BME group on Days 28, 56 and 84 as compared to placebo. Serum cortisol levels were significantly reduced from baseline to Day 56 and 84, whereas serum BDNF was significantly increased on Day 84 for BME group as compared to placebo. However, no significant effects were observed for sustained attention and planning tasks. No safety concerns were observed. **Conclusion:** BME supplementation improved memory and cognitive functions. Anxiety and serum cortisol levels were significantly reduced and sleep quality and serum BDNF was increased by BME consumption. The investigational product was safe and well tolerated throughout the study.

Trial Registration: Clinical Trials Registry, India – Identifier CTRI/2023/05/052880

Keywords: Bacopa; Memory; Cognitive function; Bacosides; Neurodegenerative diseases; Mental alertness; Alzheimer's disease

Introduction

Cognitive impairment associated with aging in otherwise healthy individuals is a major public health concern. Age-related cognitive decline varies considerably across individuals and substantially affects quality of life during old age [1-6]. Cognitive functions such as memory, attention, language, and executive functions are essential for day-to-day activities and starts declining gradually in the late 20s, and is further accelerated in case of neurodegenerative conditions such as Alzheimer's disease [7,8]. Early interventions help to maintain normal cognitive aging [9].

Cognitive enhancers, also known as nootropics, are prescribed to improve cognitive performance in elderly individuals and those with dementia. However, nootropics are increasingly being used by healthy individuals seeking to improve their attention, memory, focus, concentration, and alertness. Students use nootropics to boost attention, increased vigilance and to stay awake for an extended period [10,11]. Herbal products extensively used in traditional medicine are increasingly being used as a source of nootropics to promote cognitive functions and alleviate symptoms of memory loss and depression. Bioactive from plant extracts provide support against oxidative stress as well as improve cerebral vascular function and cognitive performance including memory and learning [12-16].

B. monnieri, known as brain tonic in traditional medicine is a perennial creeper found in the damp and marshy wetlands and is used to promote memory and learning as well as reduce anxiety and stress [17-19]. The bioactive phytochemicals present in Bacopa include a group of steroidal saponins known as Bacosides (A and B), bacopasides III, IV, V, and bacosaponins A, B, C, D, E, and F which are responsible for neuroprotection, enhance memory, and antioxidant properties [20-22]. *In vitro* and *in vivo* experimental studies have demonstrated that bacosides improve cognitive function through anti-oxidant support [23,24], modulation of neurotransmitters, increasing cerebral blood flow [25] and improve synaptic activity [26]. Numerous animal studies have demonstrated cognition-enhancing effects of Bacopa including improved motor learning [27] and memory [28]. Multiple clinical studies and meta-analysis of clinical studies on cognitive effects of Bacopa has demonstrated its ability to improve cognitive properties including attention.

The current research is a randomized, placebo-controlled study to evaluate the effect of *B. monnieri* extract (BME) on memory and cognition skills, stress, anxiety and sleep using a comprehensive objective software-based tools, validated questionnaires and biomarkers in healthy subjects.

Methods

A prospective, randomized, double-blind, placebo-controlled, parallel group study was conducted to assess the safety and efficacy of BME. Healthy male and/or female subjects aged between 18-55 years with normal cognition profile assessed by Mini-Mental State Examination (MMSE) and meeting the eligibility criteria were enrolled in the study.

The study was approved by BGS Global Institute of Medical Sciences Institutional Ethics Committee, Bengaluru, prior to the start and was conducted in accordance with the Indian Council of Medical Research (ICMR) ethical guidelines, International Council for Harmonization (ICH) Guidance on Good Clinical Practice (E6R2) and the Declaration of Helsinki. The study was registered with the Clinical Trials Registry of India (CTRI/2023/05/052880).

There were six study visits for each subject – Screening/ Baseline Visit (Day -7 to Day 0), Randomization Visit (Day 1), Three Follow-Up Visits (Day 14±3 days; Day 28±3 days; Day 56±3 days) and End of Study Visit (Day 84±3 days). During the screening period, subjects were explained about the study procedures, risks and discomforts, the investigational product, and other alternative treatments. After obtaining voluntary informed consent, demographic details, medical history including hypertension, diabetes mellitus, chronic liver, gastrointestinal, renal disorder, psychiatric disorders, surgeries, any other clinically significant medical, medication history, prior and concomitant medications were obtained. During randomization visit, eligible subjects were randomly assigned in double-blinded fashion into two treatment groups to receive placebo or BME in 1:1 ratio. The subjects were asked to consume one capsule every morning after breakfast for 84 days.

The active group (BME) capsules contained 300 mg *B. monnieri* extract (30% total bacosides) providing 90 mg of total bacosides (commercially known as B-Lit Bacopa) and the placebo capsules were made of Microcrystalline Cellulose. Both the capsules were manufactured by Samriddh Nutractive Private Limited, India.

Fasting blood samples were collected for biomarker analysis; serum Brain-Derived Neurotrophic Factor (BDNF), serum cortisol as well as safety assessments which included complete blood count (CBC), alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (TB) and alkaline phosphatase (ALP) for liver function and serum creatinine and blood urea nitrogen (BUN) for renal function. After blood sample collection, subjects were evaluated for memory and cognitive functions. All tests were administered by a single examiner. Acute effects of BME on cognitive skills were assessed by conducting tests at baseline at least 1 hour before supplement administration and at 1, 3 and 5 hours post-dose on Day 1. Anxiety levels were assessed by Beck

Anxiety Inventory (BAI) and sleep quality by Pittsburgh Sleep Quality Index (PSQI).

Creyos Cognitive Test Battery

Creyos has been used by several researchers in academic and commercial settings which uses scientifically rigorous and validated suite of cognitive tasks backed by more than 300 peer-reviewed studies and with 25+ years of scientific research. The tasks have been shown in numerous published studies to be extremely sensitive to even minor changes in cognition. The specific tasks include domains like verbal short-term memory, spatial short-term memory, visuospatial working memory, working memory, episodic memory, concentration, alertness, sustained attention, reasoning, planning and mental flexibility. Details of these tests are available on www.creyos.com.

Beck Anxiety Inventory

Beck Anxiety Inventory (BAI) is a tool for assessing the severity of an individual's anxiety symptoms. It has 21 questions that can be self-administered or given verbally by a practitioner.

Pittsburgh Sleep Quality Index (PSQI)

The Pittsburgh Sleep Quality Index (PSQI) is a self-rated questionnaire that measures sleep quality and disruptions over a one-month period. It includes 19 self-reported items and 5 additional questions for the bed partner. It is divided into seven domains: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, the use of sleeping medication, and daytime dysfunction.

Serum BDNF and cortisol was analyzed using ELISA kits by the central laboratory.

Eligibility Criteria

Inclusion Criteria

Subjects with the following criteria were included in the study. Healthy male or female adults, ≥18 to ≤55 years; subjects with body mass index of 18.5 kg/m^2 to 29.9 kg/m^2 (both limits inclusive); those who refrained from taking any medications to improve memory and cognition during the study; healthy subjects with MMSE score of 24-30; those who were willing to refrain from consuming caffeine and caffeine-containing products 12 hour prior to visiting days; those who were willing to refrain from consuming alcohol 24 hours prior to the visiting days; those who were willing to refrain from vigorous physical activity 12 hours prior to test days; those having basic computer literacy and exposure to computerized tests; those who were willing and able to give informed consent and comply with the study procedures.

Exclusion Criteria

Subjects who met any of the following criteria were excluded from the study. Subjects who were consuming any memory improving medicines, alcohol, tobacco, or any other central nervous system (CNS) acting medicines; have hypersensitivity or history of allergy to the study product; have any degree of cognitive impairment; suffer from sleep disturbances and/or were taking sleep aid medication; having chronic diseases like hypertension, ischemic heart disease, diabetes, and psychiatric or CNS disorders; those were on anxiolytics, anti-depressants, antipsychotics, anticonvulsants, centrally acting corticosteroids, opioid pain relievers, hypnotics, and/or prescribed sleep medications; with excessive habitual caffeine consumption (>300 mg caffeine/day or ≥3 cups of caffeinated coffee/day) throughout the study period; who were pregnant, nursing, or planning a pregnancy within the study participation period; those who were currently participating or having participated in another clinical trial during the last 3 months prior to the beginning of this study; and any additional condition(s) that in the investigators opinion would warrant exclusion from the study or prevent the subject from completing the study.

Endpoints Evaluation

Efficacy endpoints: Primary efficacy endpoints included mean change in verbal short-term memory assessed by Digit Span task, visuospatial working assessed by Monkey Ladder task, working memory assessed by Token Search task, episodic memory assessed by Paired Associates task, and spatial short-term memory assessed by Spatial Span task using Creyos Assessment (formerly Cambridge Brain Sciences) from baseline to Days 28, 56 and 84.

Secondary efficacy endpoints were mean change in cognitive skills parameters - alertness and sustained attention through Feature Match task and Sustained Attention to Response Task; concentration assessed by Double Trouble task; reasoning assessed by Odd One Out and Grammatical Reasoning task; planning assessed by Spatial planning task and mental flexibility assessed by Rotations task using Creyos on Day 0 (baseline) and Days 14, 28, 56 and 84. Concentration assessed by Double Trouble task, alertness and sustained attention assessed by Feature Match task and Sustained Attention to Response Task were also evaluated at 1, 3 and 5 hours post dose on Day 1 by Creyos Assessment.

Mean change in serum BDNF (baseline, Day 84), serum cortisol levels (baseline, Day 56 and 84), BAI scores for evaluation of anxiety levels, PSQI scores for evaluation of sleep quality (baseline, Days 28, 56 and 84), and safety (incidence of adverse events) from baseline to Day 84 were evaluated.

Statistical methods

Sample Size Determination

A study population of 70 subjects, 35 in each arm, was considered sufficient to detect a clinically important difference

between groups with 80% power and a 5% level of significance. Considering a dropout rate of 10% the sample size was finalized as 80 subjects. The sample size calculation was based on difference of two treatments considered to be medically relevant. Assuming for the primary endpoints, a common standard deviation of 3.80 at the end of treatment, 35 per group would be sufficient to detect a difference of 1.86 in mean difference between the 2 treatment group with power of 80% and a 0.05 two-sided level of significance.

Efficacy Evaluation

The continuous type efficacy endpoints were summarized by treatment using descriptive statistics (n [number of subjects], mean, standard deviation (SD), standard error (SE), median, minimum and maximum). Within group significance was evaluated by using paired t-test and between group significance was evaluated using independent t-test or Wilcoxon rank sum test as determined by Shapiro-Wilk normality test. A value of $p<0.05$ was considered statistically significant.

Safety Evaluation

The continuous type safety endpoints were summarized by treatment using descriptive statistics (n [number of subjects], mean, SD, SE, median, minimum and maximum) on safety population. The categorical type safety endpoints were summarized by treatment using frequency and percentage on safety population. The difference between the treatments groups were evaluated using independent t-test. The analysis was conducted on the safety population only. The safety population included all randomized subjects who received investigational products.

Results

A total of 89 subjects were screened for the study and 9 subjects were screen failure. Of the 80 randomized subjects, 36 subjects from placebo group and 38 subjects from BME group completed the study (Figure 1). The efficacy analysis included data from the study participants who completed it, while the safety analysis included data from all the randomized subjects.

Of the 74 subjects who completed the study, 50 were males and 24 were females (Table 1). Twenty-three males and 13 females in placebo group, and 27 males and 11 females in BME group completed the study. The mean body weight (±SD) of subjects were 66.93±8.77 kg in placebo group and 69.55±9.37 kg in BME group. The mean (±SD) height was 164.58±10.60 cm in the placebo and 165.55±6.81 cm in BME group. The mean (±SD) BMI of subjects in the placebo and BME groups were 24.74±2.91 kg/m^2 and 25.33±2.82 kg/m^2, respectively. The mean (±SD) MMSE score of subjects in the placebo group was 24.31±1.41 and BME group was 24.26±1.22. No significant differences were observed between the groups for any of the parameters.

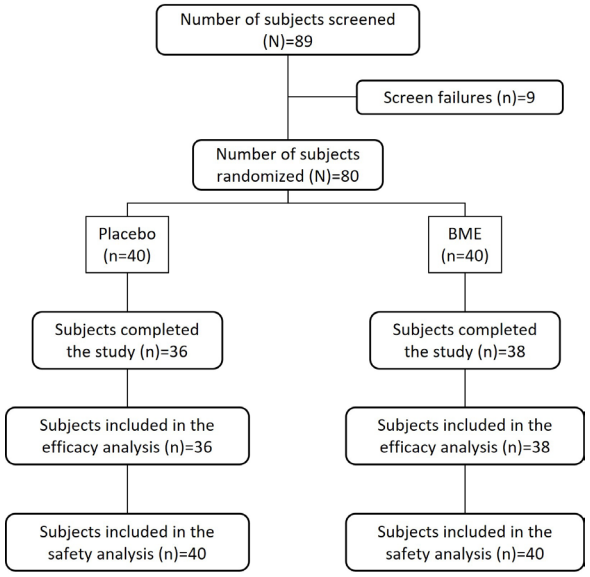

Figure 1: Consolidated Standards of Reporting Trials (CONSORT) diagram – placebo vs. *Bacopa monnieri* Extract (BME).

	Placebo (N=36)	BME (N=38)
Age (years) [Mean ± SD]	35.83±7.06	37.21±6.73
Male [n (%)]	23 (31%)	27 (36%)
Female [n (%)]	13 (18%)	11 (15%)
Weight (kg) [Mean ± SD]	66.93±8.77	69.55±9.37
Height (cm) [Mean ± SD]	164.58±10.60	165.55±6.81
BMI (kg/m^2) [Mean ± SD]	24.74±2.91	25.33±2.82
MMSE Total Score (Mean ± SD)	24.31±1.41	24.26±1.22
BME: *Bacopa monnieri* Extract; MMSE: Mini-Mental State Examination; N: Number of subjects in the specified group; n: Number of subjects in the specified category; SD: Standard deviation		

Table 1: Demographic details of participants.

Efficacy Evaluation

Primary Endpoints

The primary objective of the study was to evaluate the effect of BME versus placebo on memory from baseline to Day 84.

Verbal Short-term Memory Assessed by Digit Span Task

Summary of mean change for verbal short-term memory assessed by Digit Span Task by visit and treatment is presented in Figure 2a. For the BME group, the change from baseline in the Digit Span Task scores was significant (p<0.05) on Day 28 (BME=1.26±2.09, placebo=0.22±1.99), Day 56 (BME=1.82±2.01, placebo=0.14±2.84) and Day 84 (BME=2.45±2.57, placebo=0.17±2.48) versus placebo.

Spatial Short-term Memory Assessed by Spatial Span Task

Summary of mean change for spatial short-term memory assessed by Spatial Span Task by visit and treatment is presented in Figure 2b. For the BME group, the change from baseline for the Spatial Span Task score was non-significant on Day 28 (BME=0.34±0.99, placebo=0.17±1.28). However, the change from baseline was significant (p<0.05) on Day 56 (BME=0.79±0.91, placebo=0.17±1.21) and Day 84 (BME=1.21±1.04, placebo=0.22±1.48) when compared to the placebo.

Working Memory Assessed by Token Search Task

Summary of working memory assessed by Token Search Task by visit and treatment is presented in Figure 2c. For the BME group, the change from baseline for the Token Search Task showed an increasing trend (p=0.0533) on Day 28 (BME=0.79±1.34, placebo=0.08±1.52). However, the change from baseline was significant (p<0.05) at Day 56 (BME=1.05±1.54, placebo=0.19±1.33) and Day 84 (BME=1.55±2.02, placebo=0.25±1.38) compared to the placebo.

Visuospatial Working Assessed by Monkey Ladder Task

Summary of mean change for visuospatial working assessed by Monkey Ladder Task by visit and treatment is presented in Figure 2d. For the BME group, the change from baseline in the Monkey Ladder Task scores was non-significant on Day 28 (BME=1.05±2.32, placebo=0.28±2.09). However, the change from baseline was significant (p<0.05) on Day 56 (BME=1.37±1.76, placebo=0.22±1.27) and Day 84 (BME=2.00±2.77, placebo=0.33±1.55) as compared to the placebo.

Episodic Memory Assessed by Paired Associates Task

Summary of episodic memory assessed by Paired Associates Task by visit and treatment is presented in Figure 2e. For the BME group, the change from baseline for the Paired Associates Task scores was significant (p<0.05) on Day 28 (BME=0.66±0.99, placebo=-0.03±1.13), Day 56 (BME=0.90±1.31, placebo=0.03±0.91) and Day 84 (BME=1.50±1.62, placebo=0.14±1.29) as compared to the placebo.

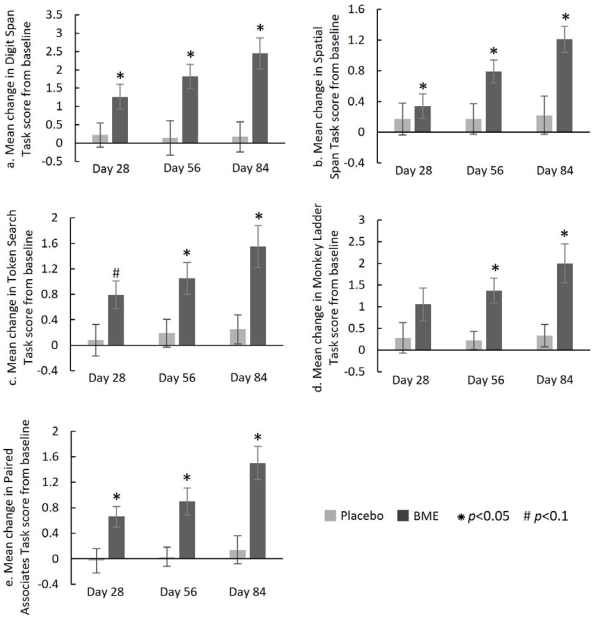

Figure 2: Summary of mean change – placebo vs. *Bacopa monnieri* Extract (BME). (a) Digit Span Task; (b) Spatial Span Task; (c) Token Search Task; (d) Monkey Ladder Task; and (e) Paired Associates Task. *p *value*<0.05; #p *value*<0.1.

Secondary Endpoints

The secondary objectives of the study was to evaluate the effect of BME on cognitive skills, anxiety, sleep quality, serum cortisol and BDNF levels from baseline to Day 84.

Cognitive Skills Parameters - Feature Match Task

Acute Effect - Featured Match Task (Baseline to 1, 3 and 5 hours Post-dose)

Summary of acute effect on alertness and sustained attention assessed by Feature Match Task by visit and treatment is presented in Figure 3a. For the BME group, the change from baseline for the Feature Match Task scores was non-significant at 1-hour post-dose (BME=15.80±34.45, placebo=15.30±35.00), 3-hour post-dose (BME=15.85±29.87, placebo=10.13±27.28) and 5-hour post-dose (BME=26.00±34.04, placebo=19.13±29.76) as compared to the placebo.

Chronic Effect - Featured Match Task (Baseline to Week 12)

Summary of chronic effect on alertness and sustained attention assessed by Feature Match Task by visit and treatment is presented in Figure 3b. For the BME group, the change from baseline for the Feature Match Task scores was significant

(p<0.05) on Day 14 (BME=21.79±31.19, placebo=5.92±24.88), Day 28 (BME=25.79±28.86, placebo=8.39±20.91) Day 56 (BME=24.42±30.15, placebo=6.44±25.36) and Day 84 (BME=27.74±26.75, placebo=7.25±26.64) as compared to the placebo.

Cognitive Skills Parameters - Sustained Attention to Response Task (SART)

Acute Effect - Sustained Attention to Response Task (Baseline to 1, 3 and 5 hours Post-dose)

Summary of acute effect on alertness and sustained attention assessed by Sustained Attention to Response Task by visit and treatment is presented in Figure 3c. For the BME group, the change from baseline for the Sustained Attention to Response Task scores was non-significant at 1-hour post-dose (BME=7.18±30.84, placebo=4.88±21.36), 3-hour post-dose (BME=13.15±21.57, placebo=5.78±12.49) and 5-hour post-dose (BME=12.65±20.75, placebo=5.18±13.83) compared to the placebo.

Chronic Effect - Sustained Attention to Response Task (Baseline to Week 12)

Summary of chronic effect on sustained attention assessed by Sustained Attention to Response Task by visit and treatment is presented in Figure 3d. For the BME group, the change from baseline for the Sustained Attention to Response Task scores was non-significant on Day 14 (BME=13.66±25.99, placebo=11.06±13.15), Day 28 (BME=13.05±24.15, placebo=6.14±17.01) Day 56 (BME=16.29±21.59, placebo=7.19±16.33) and Day 84 (BME=11.21±22.45, placebo=4.06±16.97) compared to the placebo.

Cognitive Skills Parameters - Concentration Assessed by Double Trouble Task

Acute Effect - Double Trouble Task (Baseline to 1, 3 and 5 hrs Post-dose)

Summary of acute effect on concentration assessed by Double Trouble Task by visit and treatment is presented in Figure 3e. For the BME group, the change from baseline for the Double Trouble Task scores was non-significant at 1-hour post-dose (BME=10.35±9.25, placebo=8.25±11.07). The change from baseline was significant (p<0.05) at 3-hour post-dose (BME=14.50±9.15, placebo=8.70±11.09) and 5-hour post-dose (BME=16.23±11.69, placebo=10.30±9.54) as compared to the placebo.

Chronic Effect - Double Trouble Task (from Baseline to Week 12)

Summary of chronic effect on concentration assessed by Double Trouble Task by visit and treatment is presented in Figure 3f. For the BME group, the change from baseline for the Double Trouble Task scores was significant (p<0.05) on Day 14 (BME=19.68±10.13, placebo=9.69±12.57), Day 28 (BME=22.97±11.96, placebo=16.33±12.75), Day 56 (BME=24.24±14.22, placebo=16.08±13.98) and Day 84 (BME=27.11±14.41, placebo=15.39±16.87) as compared to the placebo.

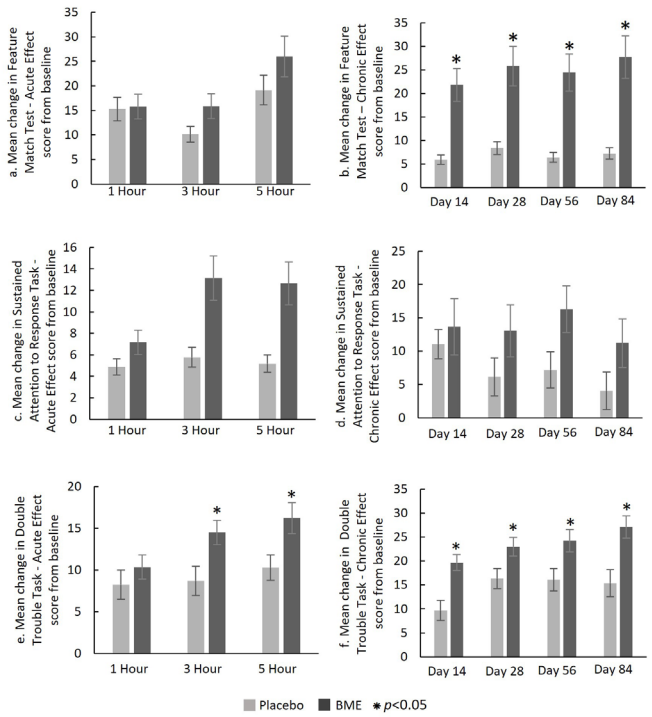

Figure 3: Summary of mean change – placebo vs. *Bacopa monnieri* Extract (BME). Feature Match Test − (a) acute effects and (b) chronic effects; Sustained Attention to Response Task−(c) acute effects and (d) chronic effects; Double Trouble Task −(e) acute effects and (f) chronic effects. *p *value*<0.05.

Odd One Out Task

Summary of reasoning assessed by Odd One Out Task by visit and treatment is presented in Figure 4a. For the BME group, the change from baseline for the Odd One Out Task scores was non-significant on Day 14 (BME=1.13±3.14, placebo= -0.08±3.94). The change from baseline was significant (p<0.05) on Day 28 (BME=0.87±3.63, placebo= -0.11±3.12) at Day 56 (BME=1.68±2.73, placebo= -0.03±3.45) and Day 84 (BME=2.08±2.58, placebo=0.03±4.55) as compared to the placebo.

Grammatical Reasoning Task

Summary of reasoning assessed by Grammatical Reasoning Task by visit and treatment is presented in Figure 4b. For the BME group, the change from baseline for the Grammatical Reasoning Task score showed an increasing trend

on Day 14 (BME=3.55±6.03, placebo=1.14±4.95; p=0.0633). The change from baseline were significant (p<0.05) on Day 28 (BME=4.47±5.80, placebo=2.47±4.00), Day 56 (BME=5.90±6.60, placebo=2.19±6.69) and Day 84 (BME=7.42±6.45, placebo=4.08±5.89) as compared to the placebo.

Spatial Planning Task

Summary of planning assessed by Spatial Planning Task by visit and treatment is presented in Figure 4c. For the BME group, the change from baseline for the Spatial Planning Task scores was non-significant on Day 14 (BME=7.45±13.70, placebo=5.14±14.32), Day 28 (BME=8.79±13.93, placebo=7.97±15.89), and Day 56 (BME=14.03±18.76, placebo=9.75±17.07), and showed an increasing trend on Day 84 (BME=16.03±15.14, placebo=10.00±16.57; p=0.0968) as compared to the placebo.

Rotations Task

Summary of mental flexibility assessed by Rotations Task by visit and treatment is presented in Figure 4d. For the BME group, the change from baseline for the Rotations Task scores was non-significant (p>0.05) at Day 14 (BME=35.61±40.41, placebo=17.28±53.24), Day 56 (BME=41.16±58.50, placebo=18.61±53.83) and an increasing trend at Day 28 (BME=33.74±48.62, placebo=19.86±46.80, p=0.0886) compared to the placebo. The change from baseline was significant (p<0.05) at Day 84 (BME=49.74±48.00, placebo=20.08±42.78) compared to the placebo.

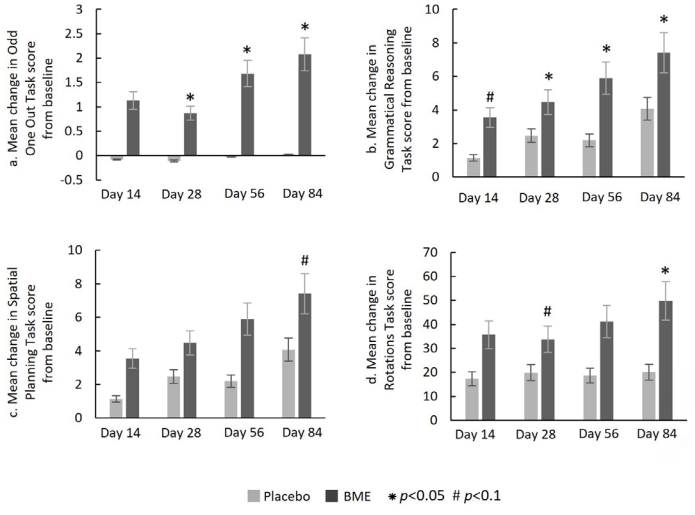

Figure 4: Summary of mean change – placebo vs. *Bacopa monnieri* Extract (BME). (a) Odd One Out scores; (b) Grammatical Reasoning scores; (c) Spatial Planning scores, and (d) Rotations Task scores. *p *value*<0.05; #p *value*<0.1.

Beck Anxiety Inventory (BAI)

Summary of anxiety assessed by BAI by visit and treatment is presented in Figure 5a. For the BME group, the change from baseline for BAI scores was significant (p<0.05) on Day 28 (BME= -1.45±1.03, placebo= -0.19±0.67), Day 56 (BME= -2.00±1.27, placebo= -0.44±0.77) and Day 84 (BME= -2.37±1.42, placebo= -0.36±1.07) as compared to the placebo.

Pittsburgh Sleep Quality Index (PSQI)

Summary of sleep quality assessed by PSQI by visit and treatment is presented in Figure 5b. For the BME group, the change from baseline for the PSQI scores were significant (p<0.05) on Day 28 (BME= -0.90±1.11, placebo= -0.17±1.13), Day 56 (BME= -1.42±1.27, placebo= -0.28±1.34) and Day 84 (BME= -1.71±1.49, placebo= -0.06±1.26) as compared to the placebo.

Serum Levels of Brain Derived Neurotrophic Factor (BDNF)

Summary of BDNF by visit and treatment is presented in Figure 5c. For the BME group, the change from baseline for the BDNF was significant (p<0.05) on Day 84 (BME=2.03±3.00 ng/ml, placebo= -0.14±1.62 ng/ml) as compared to the placebo.

Serum Cortisol

Summary of serum cortisol levels by visit and treatment is presented in Figure 5d. For the BME group, the change from baseline for the serum cortisol levels were significant (p<0.05) on Day 56 (BME= -4.84±3.98 mcg/dL, placebo=0.93±4.46 mcg/dL) and Day 84 (BME= -8.80±4.80 mcg/dL, placebo=1.20±5.40 mcg/dL) as compared to the placebo.

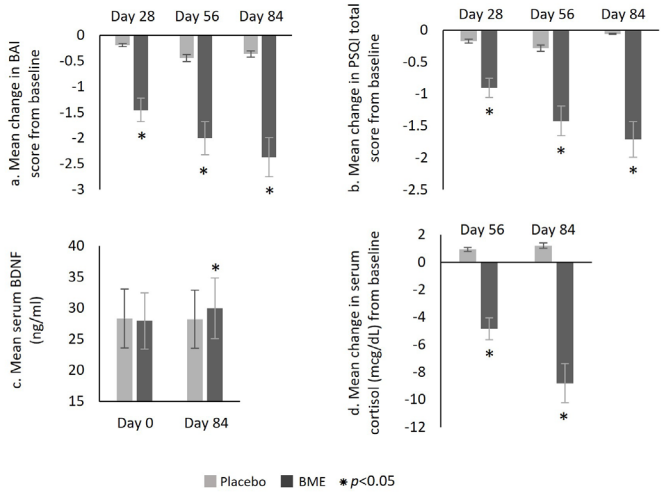

Figure 5: Mean change summary of (a) Beck Anxiety Inventory – BAI; (b) Pittsburgh sleep quality index – PSQI; (c) serum Brain Derived Neurotrophic Factor – BNDF (ng/ml), and (d) serum cortisol (mcg/dL). *p *value*<0.05.

Safety Evaluation

No clinically significant difference in the vital signs, biochemical analysis or physical parameters was found in all the groups at the end of the study as compared to the baseline. During the study, a total of 12 adverse events (AEs) were reported by 8 (19.04%) subjects. Three subjects in BME and 5 subjects in the placebo group experienced 5 (fever, headache, and body ache) and 7 (fever, headache, cold, upper respiratory tract infection (URTI), and gastritis) AEs respectively. All the AEs reported by the subjects were mild in severity and the causality of the AEs was diagnosed by the Investigator as not related to the investigational product. The outcomes of all the AEs were noted as resolved before the end of the study. None of the subjects reported any severe AE or was withdrawn from the study due to an AE or a severe AE.

Overall 8 subjects used at least one concomitant medication during the course of the study, of which 3 subjects belonged to each BME group and 5 subjects belonged to the placebo group. Most commonly used medication included acetaminophen.

Discussion

Cognitive function declines with age leading to decreased processing speed and certain aspects of memory, language, visuospatial, and executive function. The parameters of cognitive function changes vary considerably across individuals and across cognitive domains, with some cognitive functions appear more susceptible than others to the effects of aging. *B. monnieri* rich with bioactive compounds called bacosides that are known to improve cognitive properties including memory while reducing episodes of stress and anxiety [29-32]. Unlike synthetic cognitive enhancing drugs which are potentially addictive, herbal supplements are less adverse and highly tolerable by humans [13,16,18,19,33].

Here we report the results of a randomized, double-blind, placebo-controlled study where 74 out of 80 recruited subjects completed the study with 36 subjects in placebo and 38 subjects in BME group. Subjects were supplemented with 300 mg of BME that was formulated to contain 30% total bacosides (90 mg of total bacosides) or placebo for 84 days and efficacy evaluation was done on Days 1, 14, 28, 56 and 84. To our knowledge this is the most comprehensive assessment of cognitive skills along with anxiety and sleep index supported by objective biomarkers in form of BDNF and cortisol levels for *B. monnieri* in a single clinical study. Further, we also measured acute effect of BME on cognitive skills after 1, 3 and 5 hours post supplementation on Day 1.

There was significant improvement in all primary efficacy points measured for cognitive skills such as verbal and spatial short-term memory, working memory, and episodic memory from baseline to as early as Day 28 followed by Day 56 and 84 post supplementations of BME versus placebo. In case of visuospatial working memory, significant improvement was observed on Day 56 and 84 from baseline versus placebo.

Cognitive skill parameters such as alertness, concentration, reasoning and grammatical reasoning task, and mental flexibility assessed as secondary efficacy endpoints were also significantly improved from baseline on Day 84 versus placebo group. Further, we observed a significant improvement in additional secondary efficacy points such as BAI score for anxiety levels and PSQI score for sleep on Day 28, 56 and 84 from baseline in BME group versus placebo. There was also a significant increase in serum BDNF levels on Day 84 whereas significantly decreased levels of serum cortisol on Day 56 and 84 from baseline in case of BME group versus placebo.

We also measured acute effect of BME supplementation as a secondary end point on Day 1 at baseline, 1, 3 and 5 hours post-supplementation. Although we did not see a significant acute benefit in terms of improvement in alertness and sustained attention, we observed a significant improvement in concentration in case of BME group when compared against placebo at 3 and 5 hours post supplementation on Day 1.

The BME supplementation was found to be safe with no clinically significant difference in the vital signs, biochemical analysis or physical parameters and no significant adverse events observed throughout the study.

B. monnieri (BM) is an established herb in Ayurvedic medicine and has been used to promote memory, learning as well as to reduce stress and anxiety. Bacosides A and B, the active component of BM are primarily attributed to the nootropic effect which has been extensively established through in vitro and in vivo experimental studies. The BM extract improves free radical scavenging activity and antioxidant status and reduce inflammatory condition in brain tissue, modulates acetylcholine release, muscarinic cholinergic receptor binding, and choline acetylase activity which is collectively responsible for improved learning, memory, and cognitive performance [34,35]. Anti-inflammatory activity of BM extract regulate activity of microglial cell and helps in neuroprotection through β-amyloid reduction, increased cerebral blood flow to brain [32,36,37]. Further, Bacosides being nonpolar can easily cross blood-brain barrier and increase the bioavailability in the brain [38-40]. Several human clinical trials have demonstrated that BM extract promotes cognitive properties including improved immediate and delayed memory recall, processing speed, and sustained attention after supplementation for three months. [17,19,41-45] Our study results indicate significant cognitive improvements as early as Day 14 and memory improvements on Day 28 followed by 56 and 84 days supplementation which are early benefits as compared to most of the studies reported earlier.

In traditional Ayurvedic medicine, BM is considered as an adaptogenic herb and used for reducing anxiety and depression which was further supported by both experimental animal studies as well as human clinical trials [46]. In our study we measured anxiety and stress using BAI score and found significantly decreased anxiety score (BAI) as early as Day 28 and also Day 56 and 84 versus placebo thus validating the published results. Reduced stress and anxiety observed in BME was further supported in our study by the serum cortisol level which was significantly reduced on 56 and 84 days of BME supplementation. Cortisol is a hormone that regulates response to stress and found in high levels in the system during the stressful situations [47]. Our results further validate previous findings where administration of BM reduced symptoms of anxiety in subjects diagnosed with anxiety related health conditions [48,49].

BDNF, a neurotrophic factor that supports neuronal survival, growth, and neuronal plasticity and thus play an important role in promoting cognitive function [50]. Decrease in BDNF levels are seen in many neurological disorders including Alzheimer's disease [51]. Further increase in BDNF levels such as during exercise helps in ameliorating conditions associated with depression [52]. Thus, it is interesting to see significantly increased serum BDNF levels in our study after 84 days of BME supplementation which is associated with improved cognitive functions and reduced anxiety score further validating the neuroprotective role of BME.

Previously Kumar et al have demonstrated influence of Bacopa extract on hypothalamic-pituitary- adrenal (HPA) axis which further has an impact on sleep through modulation of associated neurotransmitters [53]. However, Bacopa extract supplementation did not have any effect on self-reported sleep problems in randomized, human study [54]. Contrary to the above study, we observed significant improvement in sleep quality score in our subjects from as early as Day 28 of BME supplementation which was maintained throughout the study period. It might be useful to explore further the beneficial effects of BME in subjects with sleep problems.

No significant adverse event was observed throughout the study. The safety and tolerability of the study product was confirmed by the various safety assessments in comparison with placebo. Clinical studies in children, young, and elderly people further demonstrated the suitability and benefits of BM supplementation for improving cognitive functions [29,33,55-57]. BM is generally well-tolerated. The most common side effects are gastrointestinal, including increased stool frequency, nausea, and abdominal cramps [58]. Our study did not show any side effects related to gastrointestinal or other. Future clinical studies could be designed to evaluate the effect of BME extract in subjects with cognitive impairment.

Conclusion

This is the most comprehensive study ever done on BM with extensive measures on memory, cognitive skills, anxiety, sleep, biomarkers, and acute effects. Our battery of cognitive functions test results show that the BME improves cognitive health, promotes concentration as early as 3 hours post dose on Day 1, increases overall memory, mental alertness, reasoning skills, mental flexibility, BDNF levels, sleep quality and reduces anxiety, and serum cortisol. The dosage of 300 mg BME for 86 days are well tolerated by the subjects.

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Conflict of Interest: None.

Compliance with ethics guidelines: A written approval was obtained from BGS Global Institute of Medical Sciences Institutional Ethics Committee, Bangalore, India, before commence of the study. The study was registered with the Clinical Trials Registry of India (CTRI/2023/05/052880). The study was conducted as per the regulatory requirements of the Indian Council of Medical Research, ethical guidelines, International Council for Harmonization Guidance on Good Clinical Practice (E6R2) and the Declaration of Helsinki. A voluntary informed consent was obtained, in written, from every participant before enrolling in the study.

Data Availability: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Murman DL (2015) The Impact of Age on Cognition. Semin Hear 36:111-121.

2. Farooqui T, Farooqui AA (2009) Aging: an important factor for the pathogenesis of neurodegenerative diseases. Mech Ageing Dev 130:203-215.

3. Harada CN, Love MCN, Triebel KL (2013) Normal cognitive aging. Clin Geriatr Med 29:737-752.

4. Howes MR, Perry NSL, Vásquez□Londoño C, Perry EK (2020) Role of phytochemicals as nutraceuticals for cognitive functions affected in ageing. Br J Pharmacol 177:1294-1315.

5. Glisky EL (2007) Changes in cognitive function in human aging. Brain aging: Models, methods, and mechanisms. 1:3-20.

6. Deary IJ, Corley J, Gow AJ, Harris SE, Houlihan LM, et al., (2009) Age-associated cognitive decline. Br Med Bull 92:135-152.

7. Wesnes KA, Edgar CJ (2014) The role of human cognitive neuroscience in drug discovery for the dementias. Curr Opin Pharmacol 14:62-73.

8. Jellinger KA (2023) Pathobiology of Cognitive Impairment in Parkinson Disease: Challenges and Outlooks. Int J Mol Sci 25:498.

9. Depp CA, Harmell A, Vahia IV (2012) Successful Cognitive Aging. Curr Top Behav Neurosci 10:35-50.

10. Maslen H, Faulmüller N, Savulescu J (2014) Pharmacological cognitive enhancement—how neuroscientific research could advance ethical debate. Front Syst Neurosci 8:107.

11. Normann C, Berger M (2008) Neuroenhancement: status quo and perspectives. Eur Arch Psychiatry Clin Neurosci 258:110-114.

12. Wei CC, Yu CW, Yen PL, Lin HY, Chang ST, et al., (2014) Antioxidant Activity, Delayed Aging, and Reduced Amyloid-β Toxicity of Methanol Extracts of Tea Seed Pomace from Camellia tenuifolia. J Agric Food Chem 62:10701-10707.

13. Pohl F, Lin PKT (2018) The potential use of plant natural products and plant extracts with antioxidant properties for the prevention/treatment of neurodegenerative diseases: in vitro, in vivo and clinical trials. Molecules 23:3283.

14. Dey A, Bhattacharya R, Mukherjee A, Pandey DK (2017) Natural products against Alzheimer's disease: Pharmaco-therapeutics and biotechnological interventions. Biotechnol Adv 35:178-216.

15. Zanforlin E, Zagotto G, Ribaudo G (2017) An overview of new possible treatments of Alzheimer's disease, based on natural products and semi-synthetic compounds. Curr Med Chem 24:3749-3773.

16. Dembitsky VM, Dzhemileva L, Gloriozova T, D'yakonov V (2020) Natural and synthetic drugs used for the treatment of the dementia. Biochem Biophys Res Commun 524:772-783.

17. Jain N, Tambekar O, Goel T, Bodhankar SL, Bansode DA (2024) A Comprehensive Mini Review on the Natural Product Bacopa monnieri for the Management of Alzheimer's Disease. The Natural Products Journal 14:2-13.

18. Daf MAN, Kapse MAS, Pise MSB, Makade MKP, Sawarkar MDG, Mundhada DR (2023) A Review On: Synthetic and Herbal Approaches For the Treatment of Alzheimer's Disease. [cited 2024 Jan 8].

19. Singh S, Gupta N (2024) Therapeutic Approach of Phytomedicine for Dementia: A Review. Current Traditional Medicine 10:127-142.

20. Biharee A, Chaudhari L, Bhartiya S, Kori SK, Chaudhary A, et al., (2024) A Comprehensive Study on Natural Products and their Bioactive Constituents to Cure Respiratory Diseases. The Natural Products Journal. 14:32-70.

21. Kiran NS, Yashaswini C, Lowkesh G, Range K, Madhu R (2023) Phytochemicals and Herbal Medicines: Potential Drug Candidates for Obsessive-Compulsive Disorder Treatment. In: Nutrition and Obsessive-Compulsive Disorder [Internet]. CRC Press; 2024 [cited 2024 Jan 8]. p. 189-200.

22. Sendri N, Bhandari P (2023) Bacopa monnieri. In: Herbs, Spices and their Roles in Nutraceuticals and Functional Foods 111-131.

23. Dhanasekaran M, Tharakan B, Holcomb LA, Hitt AR, Young KA, et al., (2007) Neuroprotective mechanisms of ayurvedic antidementia botanical Bacopa monniera. Phytother Res 21:965-969.

24. Russo A, Borrelli F (2005) Bacopa monniera, a reputed nootropic plant: an overview. Phytomedicine 12:305-317.

25. Aguiar S, Borowski T (2013) Neuropharmacological Review of the Nootropic Herb Bacopa monnieri. Rejuvenation Res 16:313-326.

26. Saraf MK, Prabhakar S, Pandhi P, Anand A (2008) Bacopa monniera ameliorates amnesic effects of diazepam qualifying behavioral–molecular partitioning. Neuroscience 155:476-484.

27. Prakash JC (1962) Comparative study of the effects of Brahmi (Bacopa monniera) and chlorpromazine on motor learning in rats. J Sci Industrial Research 21:93-96.

28. Singh H, Dhawan B (1997) Neuropsychopharmacological effects of the Ayurvedic nootropic Bacopa monniera Linn.(Brahmi). Indian J Pharmacol 29: S359-365.

29. Kumar N, Abichandani LG, Thawani V, Gharpure KJ, Naidu MUR, et al., (2016) Efficacy of standardized extract of Bacopa monnieri (Bacognize®) on cognitive functions of medical students: a six-week, randomized placebo-controlled trial. Evid Based Complement Alternat Med 2016:4103423.

30. Jain SK (2007) Ethnobotany and Research on Medicinal Plants in India. In: Chadwick DJ, Marsh J, editors. Novartis Foundation Symposia [Internet]. 1st ed. Wiley153-168.

31. Sivaramakrishna C, Rao CV, Trimurtulu G, Vanisree M, Subbaraju GV (2005) Triterpenoid glycosides from Bacopa monnieri. Phytochemistry 66:2719-2728.

32. Limpeanchob N, Jaipan S, Rattanakaruna S, Phrompittayarat W, Ingkaninan K (2008) Neuroprotective effect of Bacopa monnieri on beta-amyloid-induced cell death in primary cortical culture. J Ethnopharmacol 120:112-117.

33. Dave UP, Dingankar SR, Saxena VS, Joseph JA, Bethapudi B, et al., (2014) An open-label study to elucidate the effects of standardized Bacopa monnieri extract in the management of symptoms of attention-deficit hyperactivity disorder in children. Adv Mind Body Med 28:10-15.

34. Mukherjee S, Dugad S, Bhandare R, Pawar N, Jagtap S, et al., (2011) Evaluation of comparative free-radical quenching potential of Brahmi (Bacopa monnieri) and Mandookparni (Centella asiatica). Ayu 32:258-264.

35. Saraf MK, Prabhakar S, Khanduja KL, Anand A (2011) *Bacopa monniera* attenuates scopolamine-induced impairment of spatial memory in mice. Evid Based Complement Alternat Med 2011:236186.

36. Holcomb LA, Dhanasekaran M, Hitt AR, Young KA, Riggs M, et al., (2006) Bacopa monniera extract reduces amyloid levels in PSAPP mice. J Alzheimers Dis 9:243-251.

37. Rajan KE, Preethi J, Singh HK (2015) Molecular and functional characterization of *Bacopa monniera*: a retrospective review. Evid Based Complement Alternat Med 2015:945217.

38. Chakravarty AK, Sarkar T, Masuda K, Shiojima K, Nakane T, et al., (2001) Bacopaside I and II: two pseudojujubogenin glycosides from Bacopa monniera. Phytochemistry 58:553-556.

39. Pardridge WM (1999) Blood-brain barrier biology and methodology. J Neurovirol 5:556-569.

40. De K, Chandra S, Misra M (2008) Evaluation of the biological effect of brahmi (*Bacopa monnieri* Linn) extract on the biodistribution of technetium-99m radiopharmaceuticals. Life Sci J 5:45-49.

41. Cave AE, Chang DH, Münch GW, Steiner-Lim GZ (2023) A systematic review of the safety and efficacy on cognitive function of herbal and nutritional medicines in older adults with and without subjective cognitive impairment. Syst Rev 12:143.

42. Pase MP, Kean J, Sarris J, Neale C, Scholey AB, et al., (2012) The Cognitive-Enhancing Effects of Bacopa monnieri : A Systematic Review of Randomized, Controlled Human Clinical Trials. J Altern Complement Med 18:647-652.

43. Kean JD, Downey LA, Stough C (2016) A systematic review of the Ayurvedic medicinal herb Bacopa monnieri in child and adolescent populations. Complement Ther Med 29:56-62.

44. Abdul Manap AS, Vijayabalan S, Madhavan P, Chia YY, Arya A, et al., (2019) Bacopa monnieri , a Neuroprotective Lead in Alzheimer Disease: A Review on Its Properties, Mechanisms of Action, and Preclinical and Clinical Studies. Drug Target Insights 13: 1177392819866412.

45. Basheer A, Agarwal A, Mishra B, Gupta A, Srivastava MVP, et al., (2022) Use of Bacopa monnieri in the Treatment of Dementia Due to Alzheimer Disease: Systematic Review of Randomized Controlled Trials. Interact J Med Res 11: e38542.

46. Gohil KJ, Patel JA (2010) A review on Bacopa monniera: current research and future prospects. International Journal of Green Pharmacy 4:1-9.

47. Dziurkowska E, Wesolowski M (2021) Cortisol as a Biomarker of Mental Disorder Severity. J Clin Med 10:5204.

48. Singh RH, Singh L (1980) Studies on the anti-anxiety effect of the medyha rasayana drug Brahmi (Bacopa monniera Wettst). Res Ayur Siddha 1:133-148.

49. Calabrese C, Gregory WL, Leo M, Kraemer D, Bone K, et al., (2008) Effects of a Standardized Bacopa monnieri Extract on Cognitive Performance, Anxiety, and Depression in the Elderly: A Randomized, Double-Blind, Placebo-Controlled Trial. J Altern Complement Med 14:707-713.

50. Pisani A, Paciello F, Del Vecchio V, Malesci R, De Corso E, et al., (2023) The Role of BDNF as a Biomarker in Cognitive and Sensory Neurodegeneration. J Pers Med 13:652.

51. Bathina S, Das UN (2015) Brain-derived neurotrophic factor and its clinical implications. Arch Med Sci 11:1164-1178.

52. Smith MA, Makino S, Kvetnansky R, Post RM (1995) Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. J Neurosci 15:1768-1777.

53. Kumar S, Mondal AC (2016) Neuroprotective, Neurotrophic and Anti-oxidative Role of Bacopa monnieri on CUS Induced Model of Depression in Rat. Neurochem Res 41:3083-3094.

54. Lopresti AL, Smith SJ, Ali S, Metse AP, Kalns J, et al., (2021) Effects of a Bacopa monnieri extract (Bacognize®) on stress, fatigue, quality of life and sleep in adults with self-reported poor sleep: A randomised, double-blind, placebo-controlled study. Journal of Functional Foods 85:104671.

55. Barbhaiya HC, Desai RP, Saxena VS, Pravina K, Wasim P, et al., (2008) Efficacy and tolerability of BacoMind on memory improvement in elderly participants—a double blind placebo controlled study. J Pharmacol Toxicol 3:425-434.

56. Usha P, Wasim P, Joshua J, Geetharani P, Murali B, et al., (2008) BacoMind®: a cognitive enhancer in children requiring individual education programme. J Pharmacol Toxicol 3:302-310.

57. Morgan A, Stevens J (2010) Does Bacopa monnieri Improve Memory Performance in Older Persons? Results of a Randomized, Placebo-Controlled, Double-Blind Trial. J Altern Complement Med 16:753-759.

58. Walker EA, Pellegrini MV (2023) Bacopa monnieri. In: StatPearls [Internet].