Efficacy of Bacopa monnieri on memory and vascular functions: A randomised controlled trial

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Abstract

*Bacopa monnier*i (L.) Wettst. (Brahmi) is a traditional memory enhancer partly by improved cerebral blood flow. Here we sought to link improved cognitive function with better blood flow in randomised double-blinded placebo-controlled trial in an elderly cohort. Normotensive Thais, aged 55-80y having mini-mental state examination (MMSE) scores > 25, no dementia or other psycho/neurological disease, normal lipid profile, and blood biochemistry were recruited. The trial design was a 2 week run-in, 12 week intervention of test product or placebo, and 4 week washout. The intervention was an extract of *B. monnieri* leaves (eBM) in 40 ml of mulberry juice. The placebo contained mulberry juice and other constituents to match gustatory properties. End-points were a battery of memory functions, carotid blood velocity, post-ischemic microvascular blood flow, markers of vascular inflammation, blood pressure and the blood markers. Response latency was reduced by 14.2 ± 4.9% ($p = 0.022$ comparing placebo) but only in > 65s. Other memory recall parameters were either unaffected or for 'accuracy of recall' was already maximal preventing further improvement. No change was detected in carotid blood velocity while microvascular blood flow marginally increased (by 28.4 ± 8.3%, $p = 0.07$). This preliminary evidence warrant further studies on selected patients with microvascular cognitive dysfunction using more discriminating protocols.

Introduction

Dementia becomes increasingly common in aging populations of whom 50 million require full-time care costing US$1trillion globally [1,2] . As emerging economies mature, these burdens will further escalate [3] . Many more independently living elderly with milder dementia are removed from the workforce while also impinging on their quality of life. Around 50% of dementias result from cerebral small vessel disease (CSVD) and many other cases from transient ischemic attacks or from strokes [4] while ~ 70% of over 50s show brain structural changes associated with apparently silent CSVD [5] . Alzheimer's disease (AD) is the major cause for patient institutionalisation. There is increasing recognition that reduced cerebral perfusion accelerates the onset and exacerbates AD [6] , and improved life-style risks during middle age reduces dementia risk when elderly [7] . Dietary improvements are becoming the first line of treatments while nootropics have limited scope and experimental drugs targeting amyloid continue to fail [8] . Thus, treating CSVD is a necessary prelude to any direct amelioration of neuronal function. While metabolic disease is a major driver of peripheral vascular disease, the blood-brain barrier, the glial neuro-vascular unit and limited reserves of metabolic substrate create unique challenges for pharmacological therapies [9] .

Long-term dietary interventions are potentially the most effective dementia treatments, but poor adherence and a large absolute number of residual refractory cases limit their success. Alternative treatments might be plants traditionally used for improving cognitive function[10]. Among candidate herbals, *Bacopa monnieri* (L.) Wettst. or Brahmi (BM) has been an important component of Ayurvedic medicine and has traditionally been used to enhance memory. BM contains an abundance of potentially

bioactive compounds, including the saponins bacopaside I and bacoside A, a mixture of bacoside A_3, bacopaside II, jujubogenin isomer of bacopasaponin C, and bacopasaponin C. Flavonoids, essentially luteolin and apigenin are also present in BM[11,12]. BM has been used to promote mental health in general as a neurotonic and has a memory improving and neuroprotective properties[13–21]. BM increases working memory by promoting hippocampal neurogenesis[22] and reverses hippocampal neurodegeneration[23]. Such anti-dementia properties may be through reduced cerebral inflammation[24] and downregulation of inflammatory cytokines[25]. It may reduce oxidative stress by upregulating transcription factors for anti-oxidant enzyme expression[26] and eliminating dysfunctional mitochondria[27]. It reduces anxiety via cortisol[28,29] and $GABA_A$ receptors[30]. It is anti-depressant[31], anti-anhedonic[25], an anti-cholinesterase[32], anti-hyperglycaemic[33] and anti-hyperlipidaemic[34]. BM appears to exhibit no alteration in haematological and blood biochemistry parameters in rats[35] and no extra adverse events (AEs) over placebo in clinical trials[36,37].

Clinical studies on acute and chronic daily oral administration of BM show improved memory in healthy adults[38,39]. There was a similar effect on the healthy elderly, where consuming BM for 8 or 12 weeks improved memory[29,40]. In addition, BM enhanced cognitive performance in patients with AD or mild cognitive impairment (MCI)[41,42].

Apart from the above myriad of BM actions *in vivo*, most crucially, it is a vasodilator, and increases cerebral blood flow[43–45]. This implies that nootropic actions of BM result from normalising cerebrovascular blood flow that is necessary for maintaining or restoring neuronal function, yet cerebral blood flow and cognitive function are not commonly studied together.

In the present human trial, we address some outstanding questions surrounding the clinical application of BM as a memory enhancer:

i. Pathophysiological parameters, especially blood flow, are evidently important but not previously studied in consort with cognitive function. Most carotid blood-flow goes to the brain and can be measured non-invasively by ultrasonic Doppler flowmetry. However, direct measures of actual flow in cerebral small vessels are impractical, therefore post-occlusion microvascular cutaneous blood-flow was performed. We also measured markers of endothelial inflammation (vascular cell adhesion molecule-1 (VCAM-1) and asymmetric dimethylarginine (ADMA).
ii. Many BM studies have recruited young-middle aged participants in whom cerebral blood flow is optimal. In this study, participants aged 50–80 year were enrolled.
iii. Interventions packaged in large capsule are difficult to swallow[46] and further burdens the over-medicated elderly, a common cause of non-adherence. In this study the intervention was a fruit juice to improve adherence.

Results

Participant Recruitment. Recruitment was protracted (June 2018 to February 2019) reflecting high incidences of chronic disease in this population aged > 50y e.g., hypertension (65%), diabetes (23%)[47], and the low proportion of male recruits. Eighty people answered the advertisement and of these 53 fitted the inclusion criteria. A further 5 applicants were removed by the exclusion criteria leaving 48 to recruit, randomised and allocated to treatments arms.

Participant demographics. Demographics and baseline characteristics are compared between eBM and placebo groups in Table 1. Most metrics were balanced between groups except for sex, diastolic/mean blood pressure (BP), and Thai geriatric depression assessment (TGDS) score. MMSE-Thai 2002 was used for cognitive screening and mean scores were well-balanced between groups (26.3 ± 2.2 and 26.7 ± 1.9) and above optimal cut-off scores for detecting MCI (≤ 25). BP was higher in the eBM group that might influence cognitive and vascular outcomes.

The mean TGDS score was 3.3 ± 3.3 indicating no clinical depression. Applicants self-reporting chronic disease including schizophrenia, dementia, depression, liver disease, kidney disease, diabetes, cancer, stroke, hypertension and hyperlipidaemia, psychiatric or neurological conditions were excluded. The range of occupations reflect the general population in the recruitment area.

Table 1
Baseline demographics for participants (after allocation, before run-in).

Demographic variable	Total	min to max	Placebo	eBM	*p*-value*
Number of participants	45 (100%)	-	24 (53.3%)	23 (46.7%)	-
Gender					
Female, n	39 (86.7%)	-	20	19	-
Male, n	6 (13.3%)	-	4	2	-
Ages					
Age (yr), mean ± SD	62.5 ± 5.2	55 to 72	62.0 ± 5.2	63.1 ± 5.4	0.46
Group 55–64 year; mean ± SD (n)	59.1 ± 3(28)	55 to 64	59.1 ± 2.9(16)	59.3 ± 3.2 (12)	-
Group 65–80 year; mean ± SD (n)	68.2 ± 2(17)	65 to 72	68.1 ± 2.2(8)	68.3 ± 2.2(9)	-
Morphological, mean ± SD					
Height (cm)	154.1 ± 7.9	142 to 175	154.4 ± 7.8	153.8 ± 8.2	0.81
Body weight (kg)	57.8 ± 9.5	39.7 to 80.8	57.1 ± 7.6	58.7 ± 11.4	0.58
BMI (kg/m^2)	24.3 ± 3.2	18.4 to 31.6	24.0 ± 2.8	24.7 ± 3.6	0.46
Waist circumference (cm)	87.4 ± 9.5	68.6 to 114.0	86.9 ± 8.8	88.0 ± 10.5	0.72
Waist-hip ratio (WHR)	0.86 ± 0.06	0.74 to 0.97	0.87 ± 0.05	0.85 ± 0.06	0.44
Blood pressure, mean ± SD					
Systolic (mmHg)	120.3 ± 11.5	94 to 139	117.9 ± 11.9	123.1 ± 10.5	0.13
Diastolic (mmHg)	76.4 ± 8.4	62 to 98	73.5 ± 7.8	79.6 ± 8.1	0.02
Mean arterial (mmHg)	91.0 ± 8.6	72.7 to 111.7	88.3 ± 8.5	94.1 ± 7.9	0.02
Heart rate (BPM)	74.9 ± 10.7	48 to 106	74.6 ± 9.9	75.2 ± 11.8	0.86
Education					
Education (yr), mean ± SD	6.1 ± 3.6	4 to 16	5.5 ± 3.5	6.8 ± 3.7	0.25

Demographic variable	Total	min to max	Placebo	eBM	*p*-value*
Having 4 year of education, n	32	-	20	12	-
Having > 4 year of education, n	13	-	4	9	-
Occupation					
Trader, n	12	-	6	6	-
Farmer, n	15	-	7	8	-
Unskilled employee, n	11	-	7	4	-
Unemployed, n	7	-	4	3	-
MMSE [a] score, mean ± SD	26.5 ± 2.0	22 to 30	26.3 ± 2.2	26.7 ± 1.9	0.54
TGDS [b] score, mean ± SD	3.3 ± 3.3	0 to 12	4.2 ± 3.4	2.3 ± 3.0	0.06

[a]The normal range for the Thai MMSE is 25–30 (maximum possible is 30).

[b]Normal range TGDS was 0–12 (maximum 30).

* *p*-values compared eBM and placebo groups by unpaired Student's *t*-test equal variance. Baseline mean values measured before "run-in period".

AEs and dropouts. Following randomisation, 4 participants, all in the intervention group, were lost (2 withdrawn and 2 dropped out). (i) Before run-in, withdrawn because of over-range aspartate aminotransferase (AST). (ii) Another participant experienced head-aches after week 4, and after diagnosis of hypertension (> 140/90 mmHg) and prescribed an antihypertensive was withdrawn. (iii) A third participant withdrew because their employment was re-located 300km away. Another participant experiencing dyspnea after week 12 was hospitalised and in whom herpes zoster appeared later. Their data up to week12 was included in the analyses (Fig. 1). All cases were considered as not or probably not due to the intervention.

Assessment of harms. The only other AE was in a placebo group participant having hypertensive BPs while also complaining of dizziness. A home visit the same evening found the BP had normalised and the dizziness had ceased. The participant continued in the trial and subsequent BPs maintained baseline values.

Assessment of adherence. Four participants made diary entries or verbally responded by noting forgotten doses for consecutive 2 day periods (2 participants), 3 days (n = 1), and 6 days (n = 1), one in the eBM group and 3 placebo. All other bottles were returned empty.

Clinical monitoring. For weeks − 2 to 16, there were no changes in MMSE scores (see Supplementary Fig. S1 online), body morphometrics and blood pressure/heart rate that could be ascribed to the treatment (Table 1 and Supplementary Fig. S2 online). Serum biochemistry and haematology are detailed in Supplementary Table S1 online. All parameters matched well between groups. All parameters showed normal ranges except total cholesterol and LDL that tended to be over normal range especially for the placebo group. Small changes in serum alkaline phosphatase (ALP), conjugated bilirubin, and albumin pointed to a possible small reduction in liver function in the placebo group, or seasonal effects or asymptomatic infection.

Cognitive function tests and effects of eBM on memory. Response latency was expressed summing the response times of relevant tasks: word, picture, spatial and numeric recognition (Fig. 1). Treatment with eBM reduced latency for the response by ~ 7% compared to placebo treatment ($p = 0.03$ by ANOVA). A similar conclusion was reached when comparing response latencies to the week0 value, while the placebo treated participants maintained similar response latencies (Fig. 2a).

The proportion of correct responses (accuracy) with eBM remained constant, although the placebo may have improved (Fig. 2b). Placebo and eBM groups showed identical simple and choice latencies (Fig. 2c). Accuracy of choice (continuity of attention) reached ~ 98.5%, i.e., participants almost always made correct responses. This allowed only ~ 1% 'headroom' that is too little for detecting any memory improvement (Table 2). Of the four testing domains, word recognition was the slowest (2059 ± 82ms, SEMs) and least accurate (79.6 ± 1.2%) while numeric recognition the fastest (1464 ± 43ms) and most accurate (93.4 ± 1.0%). There was a distinct improvement for both groups taking placebo between baseline and 2 weeks suggesting a practice effect (Table 2).

Subanalysis of response latencies by participant age range showed that elderly participants responded to eBM by 14.2 ± 4.9% ($p < 0.034$ by ANOVA compared to placebo) but not the younger subgroup (Fig. 3). A similar digression was also seen for participants with < 4y of education (11.0 ± 4.1%; $p < 0.02$, who are also mostly elderly, compared to having > 4y of education (2.0 ± 2.4%). All data analysis as in Fig. 2.

Table 2
Raw values (means ± SDs) accrued for each task.

Parameter	Placebo (means ± SDs)			eBM (means ± SDs)		
	Baseline	Week 0	Week 12	Baseline	Week 0	Week 12
Latency, ms [a]	7648 ± 1760	6139 ± 1130	6249 ± 914	7621 ± 1703	6894 ± 1538	7173 ± 937
Accuracy, (400 max) [a]	331 ± 31	347 ± 24	361 ± 14	324 ± 50	352 ± 45	353 ± 40
Power of attention, ms	1781 ± 278	1702 ± 338	1736 ± 324	1835 ± 370	1622 ± 220	1719 ± 290
Continuity of attention. %	98.7 ± 2.3	98.8 ± 1.95	99.3 ± 1.1	97.1 ± 3.1	98.4 ± 2.3	99.1 ± 1.5

[a] summed latencies or accuracy of word, image, shape, and number.

Vascular function and eBM.

Carotid blood velocity. Mean blood velocity was 48.9 ± 10.9 cm/s (SD), ranged between 33–73 cm/s and average asymmetry between left and right arteries of 6.5 ± 4.0% (SD, max 30%) at week 0. Treatment with either eBM or placebo increased blood velocity (Fig. 4a). While there was some trend towards a greater enhancement for the eBM group, the effect failed statistical scrutiny. The maximum velocity recorded for any participant was 84cm/s, well below the threshold of 260cm/s for carotid stenosis. Subanalysis stratified by age (65–80 v. 55-64y) also showed no clear difference.

Post-occlusion reactive hyperemia. Peak post-occlusion reactive hyperaemia (PORH) was elevated in a similar fashion for the three hand areas (whole hand, fingers, and palmer hand) in the eBM group and possibly less so in the placebo group (Fig. 4b). Subanalysis by age showed no difference.

Endothelial inflammatory markers. Neither of these inflammatory markers, VCAM-1 nor ADMA were affected by the treatment or placebo (Table 3), nor did subanalysis by age reveal any actions.

Table 3
Serum concentrations of the endothelial inflammatory markers.

Endothelial inflammatory marker	Placebo (n = 24)			eBM (n = 21)			*p*-value [b]
	Control week 0	Treatment week 12	p-value [a]	Control week 0	Treatment week 12	p-value [a]	
VCAM-1, ng/ml	640 ± 170	650 ± 210	0.4	700 ± 180	710 ± 230	0.7	0.84
ADMA, ng/ml	126 ± 41	123 ± 38	0.8	117 ± 27	119 ± 31	0.7	0.4

red comparisons for eBM treatment at week 0 with week 12

[b] unpaired comparison of placebo v. eBM at week 12

Discussion

The current study was the first to use a palatable intervention of mulberry juice to administer the eBM to assess cognitive function of elderly, yet healthy participants.

The main outcome improved memory function in one domain, ie, a shorter response latency but this effect was confined to the elderly subgroup. Thus, the healthy elderly may particularly benefit from continual eBM treatment.

The current study shares common features to that of Peth-Nui et al [40], ie., protocols, BM extract containing same amounts of saponin glycosides per day, participants having similar lifestyles and socio-economic status. Yet Peth-Nui et al using a similar protocol, equipment and software obtained robust improvements in all their tested memory domains. An important difference was choice accuracy performance where the Peth-Nui et al participants scored ~ 116 correct responses out of a possible maximum score of 200. Our participants attained 99/100 correct responses leaving negligible dynamic range to see any further improvements. Similarly, simple recognition scores were 150/400 in the Peth-Nui et al study while our cohort achieved and ~ 350/400 again leaving reduced 'head space' to demonstrate improved performance. Another interesting comparison were simple/choice latencies (power of attention) for which the Peth-Nui et al cohort took ~ 2270 ms to respond compared ~ 1700 ms for our participants. Given similar conditions, we surmise that our participants already responded optimally leaving no scope for enhanced performance with eBM. Possible explanations for these diverse performances are: (i) The extensive selection criteria for blood pressure, cognitive screening, full blood analysis, and other underlying diseases ensures a more healthy cohort, and (ii) may arise from agrarian differences: the dry north-eastern uplands where food supplies are risky compared to our cohort living in the central plains with constant supplies of river water. Also, both cohorts were born > 50y ago when transport infrastructures, health-care, and education outside the cities was minimal, factors that drove the respective isolated possibly diverse cultures.

Sub-analysis of memory function for education and age were clearly associated with primary schooling and older age. These factors are linked because education for most Thais was elevated to secondary level after the older group had graduated.

For cognitive testing, the two-week run-in period showed a consistently shorter latency ($p = 0.000001$, week 2 vs week 0), greater accuracy ($p = 0.0001$), and shorter choice latency ($p = 0.03$) (Table 3) emphasising the importance of familiarisation and practice. No further practice effect after week 2 was apparent since the placebo treated group showed similar responses at week 0 and week 4. A practice effect may also explain the greater effect of the Peth-Nui et al study.

Data from PORH suggested a microvascular action of eBM while increased carotid blood velocity in both groups suggest a cohort-wide influence without any selective action of eBM. There might be two identifiable causes: (i) The base mulberry juice could exert a pharmacological action, especially via the anthocyanins estimated to be 28 mg in each dose[48] compared to > 10-fold amounts tested in human and animal trials[49]. So anthocyanins are unlikely to be a major confounder. (ii) The blood analyses showed some dyslipidemia, especially in placebos and other rather puzzling effects (see Supplementary Table S1 online). Carotid blood velocity is assumed to equate with increased cerebral perfusion assuming a

constant carotid diameter over 12 weeks. Future studies need concomitant accurate measurements of carotid diameter.

Blood levels of endothelial inflammation markers, VCAM-1 and ADMA were unchanged by treatment. In future studies, homocysteine, elevated fasting blood glucose and possibly intercellular adhesion molecule-1 (ICAM-1) may be a more responsive markers for vascular dysfunctional cognition[50] and should be measured throughout any trial.

Limitations of our study were involved:

(i) Choice of cognitive function tests: We chose the commonly used Thai-MMSE but several tools are more sensitive assessments of MCI and distinguish memory loss, aging, and vascular dementia[51] including the Addenbrooke's Cognitive Examination[52]. The inability to detect recall accuracy was a serious limitation and forgetting is far more mentally disabling. In future, the run-in period used to optimise response error rates of the cohort should be included.

(ii) Generalisability: The restricted cohort ethnicity and culture may not apply more widely and the rapid recall after the stimulus may not apply to everyday activities and quality of life.

(iii) Participant specification: Since only the ~ 9 most elderly participants showed shortening of recall latencies, only > 64y participants should be specified and then the role of vascular parameter could be more decisive.

(iv) Recruitment and allocation: Healthy elderly recruits defined by our selection criteria are scarce in Thailand including our catchment area where life-style disease dominate. Both genders were sought but only 6 males were found and randomisation allocated these 4:2 between groups that precluded subgroup analysis by sex. Because of small effect sizes and a small cohort, balancing groups for multiple characteristics is difficult with block randomisation and stratification. While most parameters were balanced between groups, BP showed disparity between groups. Accurate allocation is most optimally achieved by minimisation but still retains blinding. This method also allows a trial to begin without waiting for the whole cohort to be recruited[53].

(v) Drop-outs: A major problem with elderly participants in long trials is risk of unstable health status and high withdrawal rates independent of treatment and group imbalance.

Further studies are needed using participants having MCI aligned to the objectives and outcomes. Because MCI is a prelude to Alzheimer's that is mediated through vascular dysfunction, eBM action on PORH and carotid blood flow need refining. Studies should be all-female and the allocation stratified according to important covariants. Also, benefits to normal daily function of participant and their quality of life need to be quantifying.

In conclusion, eBM improves response latency in the more elderly participants, but the high recall accuracy comprised detecting improved recall. Nevertheless, there is sufficient evidence to warrant

studies on elderly patients with MCI using more refined protocols including improving cognitive functions to maintain their independent daily lives.

Methods

Trial Design. This study was a 12-week double-blinded, placebo-controlled randomised trial with two equal groups run in parallel preceded by 2 weeks of placebo run-in and ending by a 2-week follow-up. The main endpoint was memory function and three secondary end-endpoints, (i) carotid blood velocity, (ii) reactive hyperaemia, and (iii) endothelial inflammatory markers.

Approvals and registration. The protocol used in this study was approved by Naresuan University Ethical Committee for Human Research (NU-IRB) with IRB No. 0898/60 and the certificate COA No. 197/2018. The approval date of the protocol was 16/05/2018. The trial was registered with the Thai Clinical Trials Registry on 30/07/2020, registration ID TCTR20200730002. The study was conducted in accordance with the principles of the Declaration of Helsinki, The Belmont Report, CIOMS Guideline and the International Conference on Harmonization Good Clinical Practice guidelines.

Participant specification.

Eligibility criteria were

“A person, aged 55–80 years, who was not suffering from any diseases, including, schizophrenia, dementia, depression, liver disease, kidney disease, diabetes, cancer, stroke, hypertension and hyperlipidaemia treated with therapeutic anti-hyperlipidaemia drugs” that also appeared in the recruitment advertisement.

Inclusion criteria

55–80 years of age, Thai ethnicity, able to listen, speak and write in the Thai language, education at least to 4th -year of primary school and voluntarily signing the consent form.

Exclusion criteria

They were excluded, if they had any of the following conditions, i.e., liver disease, kidney disease, diabetes, cancer, stroke, hypertension, hyperlipidaemia treated by any antihyperlipidaemic drug, schizophrenia or any psychotic disorders, dementia or Alzheimer’s disease, depression (as diagnosed by a physician), pregnant or plan to become pregnant, taking herbal supplements or drugs which may interfere with the nervous system or study outcomes, smoking (> 10 cigarettes per a day), and attempting to lose weight. Definitions followed the Thai guidelines[54]

Criteria for the withdrawal: Participants were withdrawn if they met any of the following criteria during the study period: Receiving drugs or herbal supplements that may interfere with the nervous system or the study outcomes, diagnosed with schizophrenia or other psychotic disorder, dementia, Alzheimer’s disease

or depression by a physician, pregnancy, non-adherence to the investigational product, missing an appointment or the physical examination, experiencing high liver enzyme and/or high blood urea nitrogen (BUN), creatinine and estimated glomerular filtration rate (eGFR) serum levels, all outside the normal range, receiving injuries rendering them unable to continue the study, the participant voluntarily leaves the study, or experienced any AEs either acute and/or life-threatening or requiring inpatient hospitalisation.

The details of the selection process are illustrated in the participant flow diagram in Fig. 5.

Sample Size. The sample size was calculated using an effect size of 0.15 for the memory speed test comparing BM with placebo treatments after 12 weeks[40], power = 0.8 and alpha = 0.05[55]. A sample size of 32 participants was predicted, 16 for each arm, would detect a clinically important difference. To allow for dropouts, this was increased to at least 40 participants.

Recruitment and settings. Prospective participants were found by advertisements posted around Naresuan University and nearby health-promoting hospitals, direct contact with villagers in the University district. Recruitment took place from 1st June 2018 to 31st July 2018 and the trial was conducted from August 2018 to February 2019 at Phitsanulok, Thailand. All testing was performed on the campus of Naresuan University at the Faculty of Medical Science and the Cosmetics and Natural Products Research Center (CosNat), the Faculty of Pharmaceutical Sciences.

Applicants attended the University where a researcher explained the study aims, duration, visit times, procedures, potential harms and risks, answered questions, and signed an informed consent form.

In addition, the following screening tools and questionnaires were applied:

i. A personal and general information questionnaire,
ii. Medical history questionnaire,
iii. MMSE – Thai version 2002 (MMSE-Thai 2002)[56,57],
iv. TGDS[58]

Screening for inclusion and exclusion criteria were applied by 2 physicians and a researcher. Participants were paid expenses for attending at each visit (a fixed 500Baht, ~US$14.00).

Randomisation and allocation. Trial codes were allocated to participants at enrolment and the codes were randomised by block of four tables[59]. This was conducted by one of the researchers who also secured the allocation table and had no role in data collection. All other researchers and participants were blinded to treatments and this embargo continued to apply to withdrawn participants. The codes were broken immediately before data analysis began and after the last participant had completed their follow-up by a person who conducted the analysis.

Interventions. Preparation of the base/placebo: Frozen mulberries (Queen Sirikit Sericulture Center, Nan, Thailand) were boiled with water (1:1 w/v) and then filtered to produce mulberry juice. A liquid

chromatography/mass spectrometry (LC/MS) chromatogram and contents has been published[60] (Fig. 6). Constituents that might have pharmacological actions relevant to the current study were cyanidin 3-glucoside, cyanidin 3-rutinoside, pelagonidin3-O-rutinoside, rutin, and murusimic acid.

Extract of *B. monnieri* (eBM) and the investigational product: The aerial part of BM was collected from Phetchaburi province, Thailand, and identified by Associate Professor Wongsatit Chuakul, Faculty of Pharmacy, Mahidol University, Thailand. The voucher specimen (Phrompittayarat 001) was kept in the Pharmaceutical Botany Mahidol Herbarium, Mahidol University, Thailand. BM was extracted using 95% ethanol that contained bacoside A_3 (2.22%), bacopaside I (3.54%), bacopaside II (4.68%) bacopaside X (3.25%), and bacopasaponin C (2.34%), i.e., 16.0% total saponins, determined by high pressure liquid chromatography (Fig. 6) as previously reported[61,62].

The interventions were produced as 2 liquid formulations for oral administration:

i. Placebo: 39.6 ml was aliquoted into sterile dark glass bottles, sucralose solution (0.4 ml of 4% w/v) added, and the capped bottles pasteurised at 75^{o}C.
ii. Test product: 194 mg of Brahmi extract containing 31mg of total saponins was dissolved into 40 ml of mulberry juice/sucralose mix and then pasteurised as for the placebo.

Taste, odour, and visual discrimination between interventions: To test whether the two interventions could distinguish, a panel of 12 staff and students visually compared, and when blind-folded, smelled, tasted and drank the products in random order. None were able to distinguish the preparations.

Participants were asked to drink the intervention directly from the bottle about 30 min after breakfast at one time, replace the cap and return to the tray.

Outcomes and measurements. The primary outcome was improved working memory. Secondary outcomes were: increased carotid blood flow, post-ischemic cutaneous hyperaemia, and blood levels of endothelial cytokines. Additionally, AEs were observed.

Working memory. The Cognitive Drug Research (CDR) protocol is widely used to test a variety of drug classes on cognitive functions using subjects of varying ages, with varying health conditions and disabilities, in varying testing environments, and validated in dementias[63]. CDR has been adapted by us for Thai participants[40] as a battery of tests outlined in Fig. 1.

Participants sat in front of a laptop running Microsoft windows 10 system and no other application running, with a touch-sensitive screen in a quiet room throughout the ~ 20 min duration of the experiment. Participants responded to visual stimuli as quickly as possible by ‘pressing’ on either the “YES” or “NO” buttons. Recorded latency times were average for all trials in each test. For choice tests, each correct response incremented the score beginning from ‘0’. The final scores were corrected for random inputs.

Task1: Word recognition: Participants were sequentially presented with 15 on screen Thai ‘memory’ words for 1sec at 2sec intervals to remember. Immediately after, a randomly selected memory or novel word was displayed to which the participant pressed “YES” for a memory word or “NO” for a novel word. A correct response (“YES” or “NO” as appropriate) incremented the score. This sequence was run 15-fold and the final score expressed as a percentage (range 0-100%) and the average response latency updated (ms).

Task2: Picture recognition: This used a similar protocol and scoring but displayed 20 ‘memory’ photographs each for 1sec every 3sec in succession.

Task3: Spatial recognition: A 3x3 matrix of 5 red squares and 4 random yellow ‘memory’ squares were displayed for 1sec. Three sec later, the panel was redisplayed showing one randomly positioned yellow square and 8 red squares. If its position corresponded to one of the 4 ‘memory’ yellow squares, the participants would respond “YES”, otherwise respond with “NO”. This sequence was run 36 times. Response latencies (ms) were averaged. Correct response scores (“YES” or “NO” as appropriate) were incremented and on completion of the series, the score was corrected for random inputs.

Task4: Numeric working memory: Five digits (0–9) were randomly selected, and each displayed sequentially (1sec at 2sec intervals) for participants to memorise. Then, a random succession of 30 randomly generated single digits that invited “YES” or “NO” button presses depending on whether they were ‘memory’ or novel digits. This protocol was repeated twice more using new 5 digit sets. Mean latencies and correct responses were accumulated as above.

Simple response latency: The word “YES” was displayed 50-times at 1.0 to 3.5sec intervals and participants pressed the “YES” button as quickly as possible. Premature presses were ignored and accumulated response latencies were expressed in ms.

Choice reaction time: The words either “YES” or “NO” were randomly displayed at 1.0-3.5sec intervals and the participants had to press the corresponding “YES” or “NO” button for 50 trials. The response time (ms) and an accuracy score incremented if the response was correct.

These six raw parameters were used to create four memory domains:

i. emory latency sums immediate response times (ms) of the 4 tasks: words, images, shapes, and numeric stimuli totalling 101 tests.
ii. Quality of memory summed accuracy scores of the same 4 tasks (maximum of 100 points in the absence of any errors for each task – 400 total).
iii. Power of attention measures of attention and psychomotor information processing sum of simple (response time to appearance of stimulus) and choice response times (response time to decide between ‘yes’ or ‘no’ choice).
iv. Continuity of attention sums accuracy score of attention by calculating the combined percentage to the maximum full score of 100 from the choice reaction time.

Carotid blood velocity. The two carotids provide about 70% of cerebral blood flow and thus the common carotid blood velocity provides an approximation of total brain blood flow[64]. Carotid blood velocity was measured via a vascular Doppler probe (8MHz Nicolet Elite200, USA) connected to an in-house built ultrasound interface and digitiser. Signals were analysed with Matlab (v. 3.2) (Int. J. Engng Ed. Vol. 21, No. 4, pp. 649–667, 2005). Before testing, subjects were supine and rested for 5 minutes in the quiet room at 25 ^{o}C. The transducer probe was placed over the internal carotid arteries at 60^{o} to the vessel longitudinal axis. Ultrasonic Doppler blood flow waveforms were recorded during 5 sec breath-holds for both right and left internal carotid arteries in triplicate as peak systolic velocity (cm/s)[65,66].

Reactive hyperaemia. Reactive hyperaemia describes the increased downstream blood flow when the upstream arterial occlusion is released[67]. Participants were seated in Fowler's position in a quiet room at 23 ± 2°C, 50 ± 5% relative humidity with their right hand resting on a table with the palm facing upwards. Cutaneous microvascular blood velocity, an indicator of blood flow, was measured by laser Doppler perfusion using a Pericam PSI system (Perimed, Järfälla, Sweden) with a 1388 x 1038-pixel CCD camera and laser speckle contrast imaging and Perisoft software. Regions of interest were selected over the whole hand, the distal and intermediate phalanges, and palm. After 3min of baseline recording, a cuff around the upper arm was inflated to 50mmHg above systolic pressure for 2 min. Then the cuff was deflated and recording continued for another 3min. The peak of the PORH was measured as perfusion units and normalised by the participant's mean arterial blood pressure, PU/mmHg[68–70].

Blood biochemistries. Participants were asked to fast for 10-12hr before arrival. Venous blood (15 ml) was drawn to measure serum glycated haemoglobin (HbA_{1C}), lipid profile, calcium, liver function markers: AST, alanine transaminase (ALT) and ALP, and renal function markers: BUN, creatinine and eGFR (see Supplementary Table S1 online). These were undertaken before and after the run-in period (week0) and at week12 (Fig. 7).

Endothelial inflammatory marker. Plasma fractions from week1 and week12 blood samples were used to measure the endothelial markers of inflammation using the commercial sandwich ELISAs for human soluble VCAM-1, cat. No. KHT0601, lot 201801002, Invitrogen, and competitive ELISA for ADMA, cat. no. E-EL-0042, lot nos. 1TR97MXU4I and GQ3LHPLMVW, Elabscience, Texas USA. Figure 7 shows at what stages of the trial these measurements and procedures were conducted.

Main outcome tests: Memory, carotid ultrasonography, reactive hyperaemia. Querying AEs: Participant debriefing and AE reports, body weight (BW), WHR and blood pressure measurements. Blood tests: 10–12 hr fasting, lipid profile, fasting blood sugar, renal & liver functions. Endothelial markers measured blood levels of VCAM-1 and ADMA. Collect intervention: When the participants were given their codified intervention for following 2/4 weeks. Values obtained at the beginning of the run-in are termed 'baseline' values

Clinical monitoring

Briefing. During the participant interview following enrolment, the researcher emphasised the importance of recording AEs however trivial. For more serious AEs they might experience due to any cause, they should firstly contact their own or the trial doctor. Responsible cohabitants were also to be made aware of the participant's role in the trial.

Debriefings. A researcher asked the participants about any AEs and these were reported in their case report forms and into their diary. In their diaries, participants recorded symptoms, frequencies, symptom details, and the management of occurring AEs.

The participant diary. On enrolment, each participant was given a purpose-designed page-a-day diary. In this, participants entered the time they took the intervention, recorded their experiences of the study and their intervention product each day into a diary, and recorded any protocol variations. If they encounter an AE, they must enter all its details into the diary and contact a trial representative.

The diary also contained information about visit dates, times and venues, when to fast in preparation for a blood draw, and telephone numbers of a research team and the designated doctor for '24/7' access or emergency use.

Adherence to treatment. Adherence was maximised by: verbally stressing the need to take the intervention in the manner described, assessing any variances in consuming the intervention material was stressed at every debriefing session, inspecting the diary entries, and visually inspecting the returned bottles for missing contents.

Clinical parameters. Blood chemistry was measured at 3 time points (see Supplementary Table S1 online), and blood pressure and cognitive function by the MMSE-Thai were measured at every visit (see Supplementary Fig. S1 online and Supplementary Fig. S2 online).

Routine home visits were deemed unnecessarily invasive because: (a) the intervention is commonly consumed as a food or a herbal medication, (b) eBM is a much researched supplement, and (c) most elderly in our catchment area cohabit (94%) and have family caregivers (99%)[47].

Participant flow. Participants made 6 visits to the campus and entered into three phases of the trial: run-in, intervention, and follow-up (Fig. 5 and Fig. 7)

Run-in period: Placebo was consumed by all participants to: (i) ensure the participants could reliably take the intervention and cope with the testing throughout the trial, (ii) maintain their health status thus minimising dropouts, (iii) provide a practice session for memory testing, the primary endpoint, and (iv) provide two sets blood biochemistry baseline values some of which are notoriously variable.

Data analyses. Endpoint data was presented and analysed using data normalised to week 0 using the equation, $D=((d_t/d_0)-1)*100\%$ where d_t are data at weeks 4 to 16 and d_0 week 0. Unpaired *t*-test between eBM and placebo treatments at each measurement time and Tukey post hoc testing using GraphPad Prism for Windows version 5, (GraphPad Software Inc., La Jolla, CA, USA). Treatment groups (eBM v.

placebo) were compared using 2-factor repeated measures ANOVA as time-indexed rows in Graphpad at time points 4, 8, and 12 weeks. Comparisons were made between groups and week 0 tests. Values are expressed as means ± SDs or means ± SEMs as appropriated. A *p*-value < 0.05 was considered significant. 'n' refers to the number of participants relevant to test.

Abbreviations

ADMA	asymmetric dimethylarginine
AEs	adverse events
ALP	alkaline phosphatase
ALT	alanine transaminase
AST	aspartate aminotransferase
BM	Brahmi
BP	blood pressure
BUN	blood urea nitrogen
BW	body weight
CDR	Cognitive Drug Research
CSVD	cerebral small vessel disease
eBM	extract of *B. monnieri*
eGFR	estimated glomerular filtration rate
FBG	fasting blood sugar
HbA1C	glycated haemoglobin
LC/MS	liquid chromatography/mass spectrometry
MCI	mild cognitive impairment
MMSE	mini-mental state examination
PORH	post-occlusion reactive hyperaemia
TGDS	Thai geriatric depression assessment

VCAM-1 vascular cell adhesion molecule-1

WHR waist-hip ratio

Declarations

Data available

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions statement

Conceptualization, K.I., N.W. and K.C.; methodology and experimental design, N.K, K.I., N.W., W.K., C.W., O.K., P.T., D.R. and K.C.; software, J.W., N.W. and W.K.; validation, N.K and K.C.; formal analysis, N.K, C.N.S., W.K. and K.C.; investigation, N.K., W.K., C.W., O.K., P.T., D.R., N.N., S.W., U.C. and A.I.; resources, K.I, N.W and K.C.; data curation and interpretation, C.N.S., N.K and K.C.; writing—original draft preparation, N.K.; manuscript writing—review and editing, C.N.S. and K.C.; visualization, K.I, N.W and K.C.; supervision, K.I, N.W and K.C.; project administration, K.C.; funding acquisition, K.C.

Competing interests

All authors declared no conflict of interest.

Additional information

Supplementary Information.

Availability of Data and Materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

References

1. World-Health-Organization. Global action plan on the public health response to dementia 2017-2025. (World Health Organization, Geneva, 2017). https://apps.who.int/iris/bitstream/handle/10665/259615/9789241513487-eng.pdf?sequence=1 (accessed 1 August 2022)
2. Alzheimer's-Disease-International. World Alzheimer Report 2019: Attitudes to dementia. (Alzheimer's Disease International, London, 2019). https://www.alzint.org/u/WorldAlzheimerReport2019.pdf (accessed 1 August 2022)
3. Raju, M., Gopi, V. P., Anitha, V. & Wahid, K. A. Multi-class diagnosis of Alzheimer's disease using cascaded three dimensional-convolutional neural network. *Physical and Engineering Sciences in Medicine*, 1-10 https://doi.org/10.1007/s13246-020-00924-w (2020).
4. Zotin, M. C. Z., Sveikata, L., Viswanathan, A. & Yilmaz, P. Cerebral small vessel disease and vascular cognitive impairment: from diagnosis to management. *Current Opinion in Neurology* **34**, 246-257. doi: 10.1097/WCO.0000000000000913. https://pubmed.ncbi.nlm.nih.gov/33630769/ (2021).
5. Caunca, M. R., Leon-Benedetti, D., Latour, L., Leigh, R. & Wright, C. B. Neuroimaging of cerebral small vessel disease and age-related cognitive changes. *Frontiers in aging neuroscience* **11**, 145. https://doi.org/10.3389/fnagi.2019.00145 (2019).
6. Gorelick, P. B. *et al.* Defining optimal brain health in adults: a presidential advisory from the American Heart Association/American Stroke Association. *Stroke* **48**, e284-e303. https://doi.org/10.1161/STR.0000000000000148 (2017).
7. Flicker, L. Life style interventions to reduce the risk of dementia. *Maturitas* **63**, 319-322. https://doi.org/10.1016/j.maturitas.2009.06.008 (2009).
8. Pan, Y., Li, H., Wardlaw, J. M. & Wang, Y. A new dawn of preventing dementia by preventing cerebrovascular diseases. *bmj* **371**. https://doi.org/10.1136/bmj.m3692 (2020).
9. Yassine, H. N. & Finch, C. E. APOE alleles and diet in brain aging and Alzheimer's disease. *Frontiers in Aging Neuroscience* **12**. https://doi.org/10.3389/fnagi.2020.00150 (2020).
10. Ratheesh, G. *et al.* Role of medicinal plants in neurodegenerative diseases. *Biomanufacturing Reviews* **2**, 1-16. https://doi.org/10.1007/s40898-017-0004-7 (2017).
11. Nuengchamnong, N., Sookying, S. & Ingkaninan, K. LC-ESI-QTOF-MS based screening and identification of isomeric jujubogenin and pseudojujubogenin aglycones in *Bacopa monnieri* extract. *Journal of pharmaceutical and biomedical analysis* **129**, 121-134. https://doi.org/10.1016/j.jpba.2016.06.052 (2016).
12. Saesong, T., Nangngam, P. & Ingkaninan, K. Pharmacognostic and physico-chemical investigations of the aerial part of *Bacopa monnieri* (L.) Wettst. *Songklanakarin Journal of Science and Technology* **41**, 397-404. https://www.thaiscience.info/Journals/Article/SONG/10993256.pdf (2019).
13. Charoenphon, N. *et al.* Brahmi (*Bacopa monnieri*): Up-to-date of memory boosting medicinal plant: A review. *Indian Journal Of Agricultural Research* **50**, 1-7, doi:10.18805/ijare.v50i1.8582. https://arccjournals.com/journal/indian-journal-of-agricultural-research/A-202 (2016).

14. Mathur, D., Goyal, K., Koul, V. & Anand, A. The molecular links of re-emerging therapy: A review of evidence of Brahmi (*Bacopa monniera*). *Frontiers in pharmacology* **7**, 44. https://doi.org/10.3389/fphar.2016.00044 (2016).
15. Abdul Manap, A. S. *et al. 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**, 1-13. https://doi.org/10.1177/1177392819866412 (2019).
16. Sukumaran, N. P., Amalraj, A. & Gopi, S. Neuropharmacological and cognitive effects of *Bacopa monnieri* (L.) Wettst – A review on its mechanistic aspects. *Complementary Therapies in Medicine* **44**, 68-82. https://doi.org/10.1016/j.ctim.2019.03.016 (2019).
17. Chaudhari, K. S., Tiwari, N. R., Tiwari, R. R. & Sharma, R. S. Neurocognitive Effect of Nootropic Drug Brahmi (*Bacopa monnieri*) in Alzheimer's Disease. *Annals of neurosciences* **24**, 111-122. https://doi.org/10.1159/000475900 (2017).
18. Le, X. T. *et al.* Protective effects of *Bacopa monnieri* on ischemia-induced cognitive deficits in mice: the possible contribution of bacopaside I and underlying mechanism. *Journal of ethnopharmacology* **164**, 37-45. https://doi.org/10.1016/j.jep.2015.01.041 (2015).
19. Stough, C. *et al.* The chronic effects of an extract of *Bacopa monniera* (Brahmi) on cognitive function in healthy human subjects. *Psychopharmacology* **156**, 481-484. https://doi.org/10.1007/s002130100815 (2001).
20. Roodenrys, S. *et al.* Chronic effects of Brahmi (*Bacopa monnieri*) on human memory. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **27**, 279-281. https://doi.org/10.1016/S0893-133X(01)00419-5 (2002).
21. Vohora, D., Pal, S. & Pillai, K. Protection from phenytoin-induced cognitive deficit by *Bacopa monniera*, a reputed Indian nootropic plant. *Journal of ethnopharmacology* **71**, 383-390. https://doi.org/10.1016/S0378-8741(99)00213-5 (2000).
22. Pham, H. T. N. *et al. Bacopa monnieri* (L.) wettst. extract improves memory performance via promotion of neurogenesis in the hippocampal dentate gyrus of adolescent mice. *International journal of molecular sciences* **21**, 3365. https://doi.org/10.3390/ijms21093365 (2020).
23. Kumar, S. S., Saraswathi, P. & Vijayaraghavan, R. Effect of *Bacopa monniera* on cold stress induced neurodegeneration in hippocampus of wistar rats: a histomorphometric study. *Journal of clinical and diagnostic research: JCDR* **9**, AF05 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4347061/ (2015).
24. Saini, N., Singh, D. & Sandhir, R. *Bacopa monnieri* prevents colchicine-induced dementia by anti-inflammatory action. *Metabolic brain disease* **34**, 505-518. https://doi.org/10.1007/s11011-018-0332-1 (2019).
25. Micheli, L. *et al. Bacopa monnieri* as augmentation therapy in the treatment of anhedonia, preclinical and clinical evaluation. *Phytotherapy Research* **34**, 2331-2340. https://doi.org/10.1002/ptr.6684 (2020).

26. Dwivedi, S. *et al.* Standardized extract of *Bacopa monniera* attenuates okadaic acid induced memory dysfunction in rats: effect on Nrf2 pathway. *Evidence-based complementary and alternative medicine* **2013**, 1-18. https://doi.org/10.1155/2013/294501 (2013).
27. Saha, S. *et al. Bacopa monnieri* inhibits apoptosis and senescence through mitophagy in human astrocytes. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* **141**, 111367. https://doi.org/10.1016/j.fct.2020.111367 (2020).
28. Murugaiyan, S. M. & Bhargavan, R. *Bacopa monnieri* alleviates aluminium chloride-induced anxiety by regulating plasma corticosterone level in Wistar rats. *Journal of Basic and Clinical Physiology and Pharmacology* **32**, 1-8. https://doi.org/10.1515/jbcpp-2019-0379 (2020).
29. Cicero, A. F. *et al.* Short-term impact of a combined nutraceutical on cognitive function, perceived stress and depression in young elderly with cognitive impairment: A pilot, double-blind, randomized clinical trial. *The journal of prevention of Alzheimer's disease* **4**, 12-15. http://dx.doi.org/10.14283/jpad.2016.110 (2017).
30. Gupta, G. L. & Sharma, L. *Bacopa monnieri* abrogates alcohol abstinence-induced anxiety-like behavior by regulating biochemical and Gabra1, Gabra4, Gabra5 gene expression of GABAA receptor signaling pathway in rats. *Biomedicine & Pharmacotherapy* **111**, 1417-1428. https://doi.org/10.1016/j.biopha.2019.01.048 (2019).
31. Mannan, A., Abir, A. B. & Rahman, R. Antidepressant-like effects of methanolic extract of *Bacopa monniera* in mice. *BMC complementary and alternative medicine* **15**, 1-8. https://doi.org/10.1186/s12906-015-0866-2 (2015).
32. Dhanasekaran, M. *et al.* Neuroprotective mechanisms of ayurvedic antidementia botanical *Bacopa monniera*. *Phytotherapy Research* **21**, 965-969. https://doi.org/10.1002/ptr.2195 (2007).
33. Udhaya Lavinya, B. & Sabina, E. P. Anti-hyperglycaemic effect of Brahmi (*Bacopa monnieri* L.) in streptozotocininduced diabetic rats: A study involving antioxidant, biochemical and haematological parameters. **7**, 531-534. https://www.cabdirect.org/globalhealth/abstract/20153415592 (2015).
34. Kamesh, V. & Sumathi, T. Antihypercholesterolemic effect of *Bacopa monniera* Linn. on high cholesterol diet induced hypercholesterolemia in rats. *Asian Pacific journal of tropical medicine* **5**, 949-955. https://doi.org/10.1016/S1995-7645(12)60180-1 (2012).
35. Sireeratawong, S., Jaijoy, K., Khonsung, P., Lertprasertsuk, N. & Ingkaninan, K. Acute and chronic toxicities of *Bacopa monnieri* extract in Sprague-Dawley rats. *BMC complementary and alternative medicine* **16**, 249-249. https://doi.org/10.1186/s12906-016-1236-4 (2016).
36. Mitra-Ganguli, T. *et al.* A randomized, double-blind study assessing changes in cognitive function in Indian school children receiving a combination of *Bacopa monnieri* and micronutrient supplementation vs. placebo. *Frontiers in pharmacology* **8**, 678. https://doi.org/10.3389/fphar.2017.00678 (2017).
37. Prabhakar, S. *et al.* Efficacy of *Bacopa monnieri* (Brahmi) and donepezil in Alzheimer's disease and mild cognitive impairment: A randomized double-blind parallel Phase 2b study. *Annals of Indian*

Academy of Neurology **23**, 767. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7900738/ (2020).

38. Kumar, N. *et al.* 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. https://doi.org/10.1155/2016/4103423 (2016).
39. Benson, S. *et al.* An acute, double-blind, placebo-controlled cross-over study of 320 mg and 640 mg doses of *Bacopa monnieri* (CDRI 08) on multitasking stress reactivity and mood. *Phytotherapy research : PTR* **28**, 551-559. https://doi.org/10.1002/ptr.5029 (2014).
40. Peth-Nui, T. *et al.* Effects of 12-week *Bacopa monnieri* consumption on attention, cognitive processing, working memory, and functions of both cholinergic and monoaminergic systems in healthy elderly volunteers. *Evid Based Complement Alternat Med* **2012**, 606424. https://doi.org/10.1155/2012/606424 (2012).
41. Dimpfel, W., Schombert, L. & Biller, A. Psychophysiological effects of Sideritis and *Bacopa* Extract and three combinations thereof—a quantitative EEG study in subjects suffering from mild cognitive impairment (MCI). *Advances in Alzheimer's Disease* **05**, 1-22, doi:10.4236/aad.2016.51001. https://www.scirp.org/html/1-2440119_64876.htm (2016).
42. Goswami, S. *et al.* Effect of *Bacopa monnieri* on cognitive functions in Alzheimer's disease patients. *International Journal of Collaborative Research on Internal Medicine and Public Health* **3**, 285-293. https://www.iomcworld.org/abstract/effect-of-bacopa-monnieri-on-cognitive-functions-in-alzheimers-disease-patients-18564.html (2011).
43. Srimachai, S. *et al. Bacopa monnieri* extract increases rat coronary flow and protects against myocardial ischemia/reperfusion injury. *BMC complementary and alternative medicine* **17**, 1-10. https://doi.org/10.1186/s12906-017-1637-z (2017).
44. Kamkaew, N., Paracha, T. U., Ingkaninan, K., Waranuch, N. & Chootip, K. Vasodilatory effects and mechanisms of action of *Bacopa monnieri* active compounds on rat mesenteric arteries. *Molecules (Basel, Switzerland)* **24**, 2243. https://doi.org/10.3390/molecules24122243 (2019).
45. Kamkaew, N., Norman Scholfield, C., Ingkaninan, K., Taepavarapruk, N. & Chootip, K. *Bacopa monnieri* increases cerebral blood flow in rat independent of blood pressure. *Phytotherapy Research* **27**, 135-138. https://doi.org/10.1002/ptr.4685 (2013).
46. Sunata, K. *et al.* Analysis of clinical outcomes in elderly patients with impaired swallowing function. *PLOS ONE* **15**, e0239440. https://doi.org/10.1371/journal.pone.0239440 (2020).
47. Taburee, W. *et al.* Health-related problems and drivers of health-related quality of life among community-dwelling older adults. *Journal of Primary Care & Community Health* **11**, 1-8. https://doi.org/10.1177/2150132720913724 (2020).
48. Chaiwong, S. *et al.* Dried mulberry fruit ameliorates cardiovascular and liver histopathological changes in high-fat diet-induced hyperlipidemic mice. *Journal of traditional and complementary medicine* **11**, 356-368. https://doi.org/10.1016/j.jtcme.2021.02.006 (2021).
49. Khoo, H. E., Azlan, A., Tang, S. T. & Lim, S. M. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & nutrition research* **61**.

https://doi.org/10.1080/16546628.2017.1361779 (2017).

50. Lai, M. M. *et al.* Relationship of established cardiovascular risk factors and peripheral biomarkers on cognitive function in adults at risk of cognitive deterioration. *Journal of Alzheimer's Disease* **74**, 163-171. https://pubmed.ncbi.nlm.nih.gov/32007955/ (2020).
51. Grundman, M. *et al.* Mild cognitive impairment can be distinguished from Alzheimer disease and normal aging for clinical trials. *Archives of neurology* **61**, 59-66. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5067353/ (2004).
52. Charernboon, T., Jaisin, K. & Lerthattasilp, T. The Thai version of the Addenbrooke's cognitive examination III. *Psychiatry Investigation* **13**, 571. https://doi.org/10.4306/pi.2016.13.5.571 (2016).
53. Lin, Y., Zhu, M. & Su, Z. The pursuit of balance: an overview of covariate-adaptive randomization techniques in clinical trials. *Contemporary clinical trials* **45**, 21-25. https://doi.org/10.1016/j.cct.2015.07.011 (2015).
54. Sueblinwong, T., Mahaisavariya, P. & Panichkul, S. The ethical guidelines for research on human subject in Thailand*. In Forum for Ethical Review Committees in Thailand*. http://www.fercit.org/file/Guideline_English_version.pdf (2007).
55. Sakpal, T. V. Sample size estimation in clinical trial. *Perspectives in clinical research* **1**, 67-69. https://link.gale.com/apps/doc/A242756815/HRCA?u=anon~d11d4291&sid=googleScholar&xid=ec32998a (2010).
56. Mf, F. Folstein SE. McHugh PR."Mini-mental state." A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr res* **12**, 189-198. https://cir.nii.ac.jp/crid/1571417125069760128 (1975).
57. Taemeeyapradit, U., Udomittipong, D. & Tepparak, N. Characteristics of behavioral and psychological symptoms of dementia, severity and levels of distress on caregivers. *Journal of the Medical Association of Thailand= Chotmaihet thangphaet* **97**, 423-430. https://europepmc.org/article/med/24964685 (2014).
58. Poungvarin, N., Prayoonwiwat, N., Devahastin, V. & Viriyavejakul, A. Dementia in Thai stroke survivors: analysis of 212 patients. *Journal of the Medical Association of Thailand = Chotmaihet thangphaet* **78**, 337-343. https://pubmed.ncbi.nlm.nih.gov/7658177/ (1995).
59. Altman, D. G. & Bland, J. M. How to randomise. *Bmj* **319**, 703-704. https://doi.org/10.1136/bmj.319.7211.703 (1999).
60. Minale, G. *et al.* Characterization of Metabolites in Plasma, Urine and Feces of Healthy Participants after Taking Brahmi Essence for Twelve Weeks Using LC-ESI-QTOF-MS Metabolomic Approach. *Molecules (Basel, Switzerland)* **26**, 2944. https://doi.org/10.3390/molecules26102944 (2021).
61. Phrompittayarat, W. *et al.* Determination of pseudojujubogenin glycosides from Brahmi based on immunoassay using a monoclonal antibody against bacopaside I. *Phytochemical analysis : PCA* **18**, 411-418. https://doi.org/10.1002/pca.996 (2007).
62. Phrompittayarat, W. *et al.* An enzyme-linked immunosorbant assay using polyclonal antibodies against bacopaside I. *Analytica chimica acta* **584**, 1-6. https://doi.org/10.1016/j.aca.2006.11.017

(2007).

63. Wesnes, K. A. Assessing change in cognitive function in dementia: The relative utilities of the Alzheimer's Disease Assessment Scale–cognitive subscale and the Cognitive Drug Research system. *Neurodegenerative Diseases* **5**, 261-263. https://doi.org/10.1159/000113719 (2008).
64. Zarrinkoob, L. *et al.* Blood flow distribution in cerebral arteries. *Journal of Cerebral Blood Flow & Metabolism* **35**, 648-654. https://doi.org/10.1038/jcbfm.2014.241 (2015).
65. Lee, W. General principles of carotid Doppler ultrasonography. *Ultrasonography* **33**, 11-17. https://doi.org/10.14366/usg.13018 (2014).
66. Gerhard-Herman, M. *et al.* Guidelines for noninvasive vascular laboratory testing: a report from the American Society of Echocardiography and the Society for Vascular Medicine and Biology. *Vascular medicine* **11**, 183-200. https://doi.org/10.1177/1358863x06070516 (2006).
67. de Mul, F. F., Blaauw, J., Smit, R. J., Rakhorst, G. & Aarnoudse, J. G. Time development models for perfusion provocations studied with laser-Doppler flowmetry, applied to iontophoresis and PORH. *Microcirculation (New York, N.Y. : 1994)* **16**, 559-571, doi:10.1080/10739680902956107. https://pubmed.ncbi.nlm.nih.gov/19488922/ (2009).
68. Roustit, M. & Cracowski, J. L. Non-invasive assessment of skin microvascular function in humans: an insight into methods. *Microcirculation (New York, N.Y. : 1994)* **19**, 47-64. https://doi.org/10.1111/j.1549-8719.2011.00129.x (2012).
69. Petrofsky, J. *et al.* The effect of moist air on skin blood flow and temperature in subjects with and without diabetes. *Diabetes technology & therapeutics* **14**, 105-116. https://doi.org/10.1089/dia.2011.0128 (2012).
70. Cordovil, I. *et al.* Evaluation of systemic microvascular endothelial function using laser speckle contrast imaging. *Microvascular research* **83**, 376-379. https://doi.org/10.1016/j.mvr.2012.01.004 (2012).

Figures

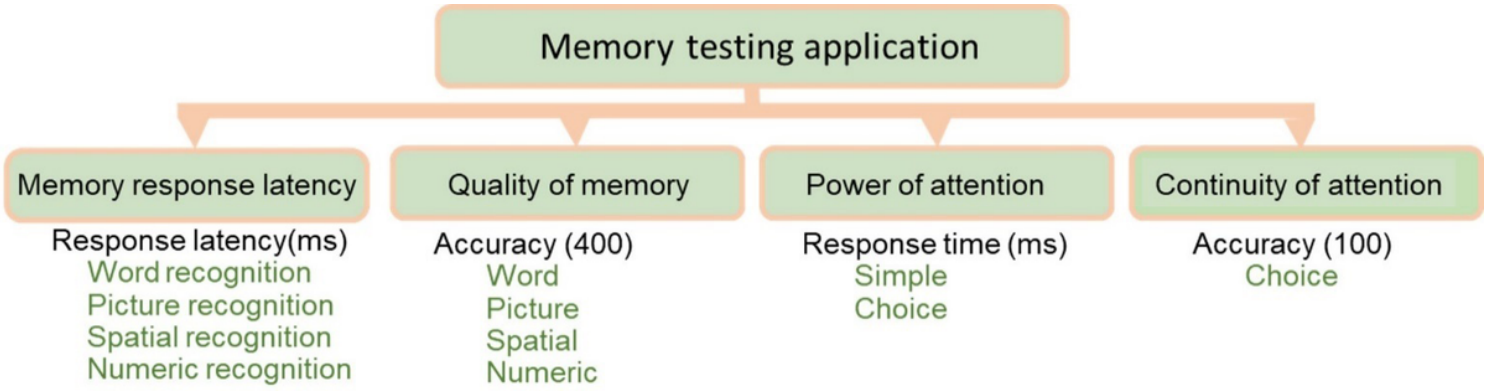

Figure 1

The Cognitive Drug Research (CDR) testing scheme.

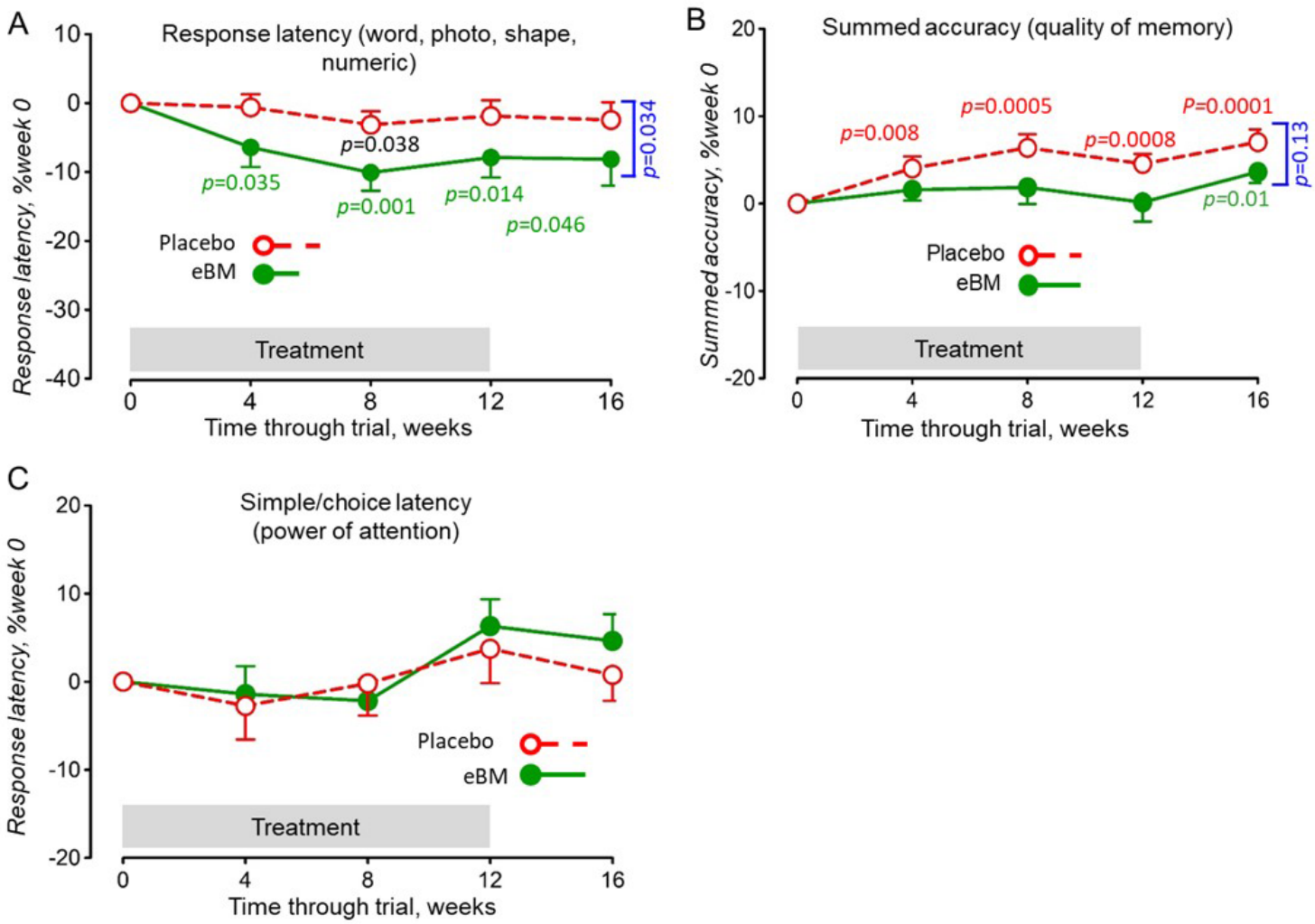

Figure 2

Cognitive function tests: (a) Summed latencies to words, images, spatial, and numeric stimuli, (b) Quality of memory by summed accuracy (%) of responses to words, images, spatial, and numeric stimuli; and (c) Power of attention as simple and choice response times. The value for each data point is expressed as $((d_t/d_0)-1)*100\%$ where d_t/d_0 are data values at weeks n and 0 and shown as means±SEM. *P*-values in green compare week n with week 0 by paired tests for eBM or red for placebo treated participants. P-values in black make unpaired comparisons between eBM vs placebo at the same time point. 2-way ANOVA comparisons of eBM vs placebo for 4, 8, and 12 week time points yielded p-values in blue.

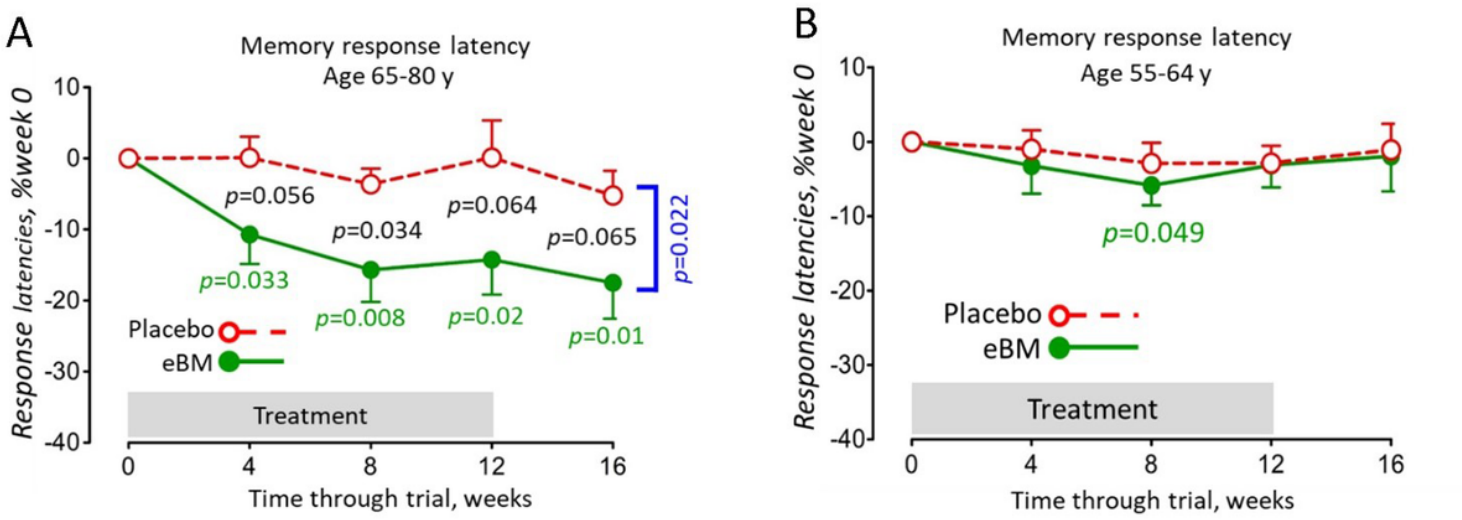

Figure 3

Sub-group analysis of response latency in elderly (A) and less elderly participants (B). Analyses as figure 2.

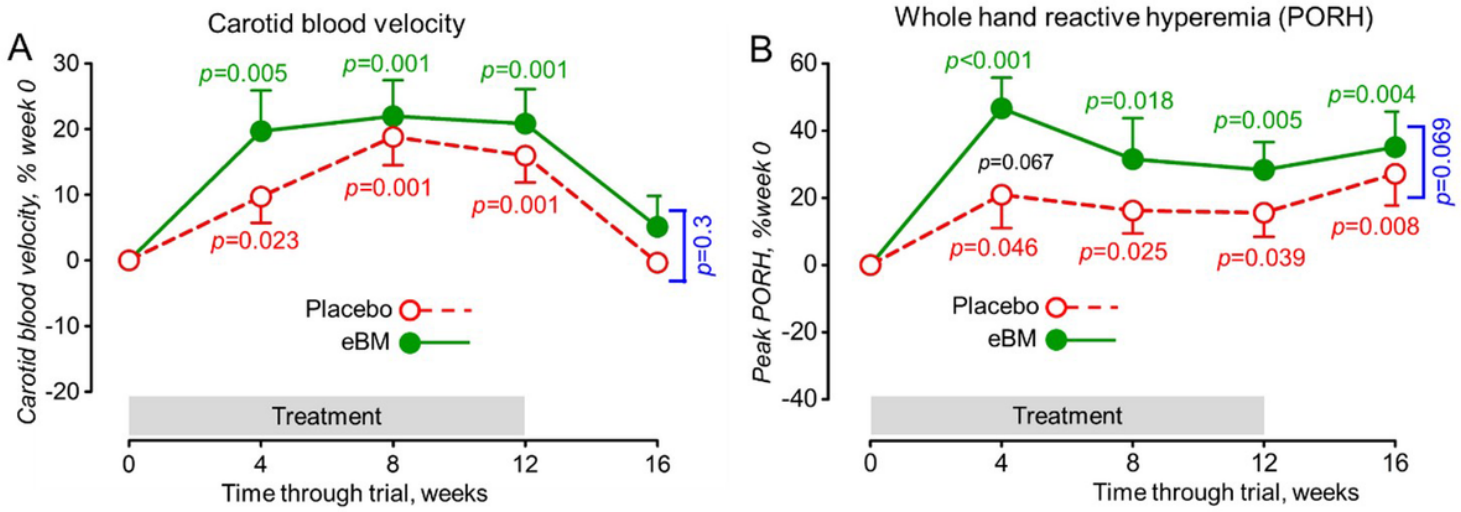

Figure 4

Blood flow measured as (A) blood velocity in both carotid arteries by ultrasonography and (B) peak post-occlusion reactive hyperaemia (PORH) in the palmer aspect of the right hand following occlusion of the brachial artery. C and D, PORH in younger (55-64y) and older (65-80y) participants. Analyses as figure 2.

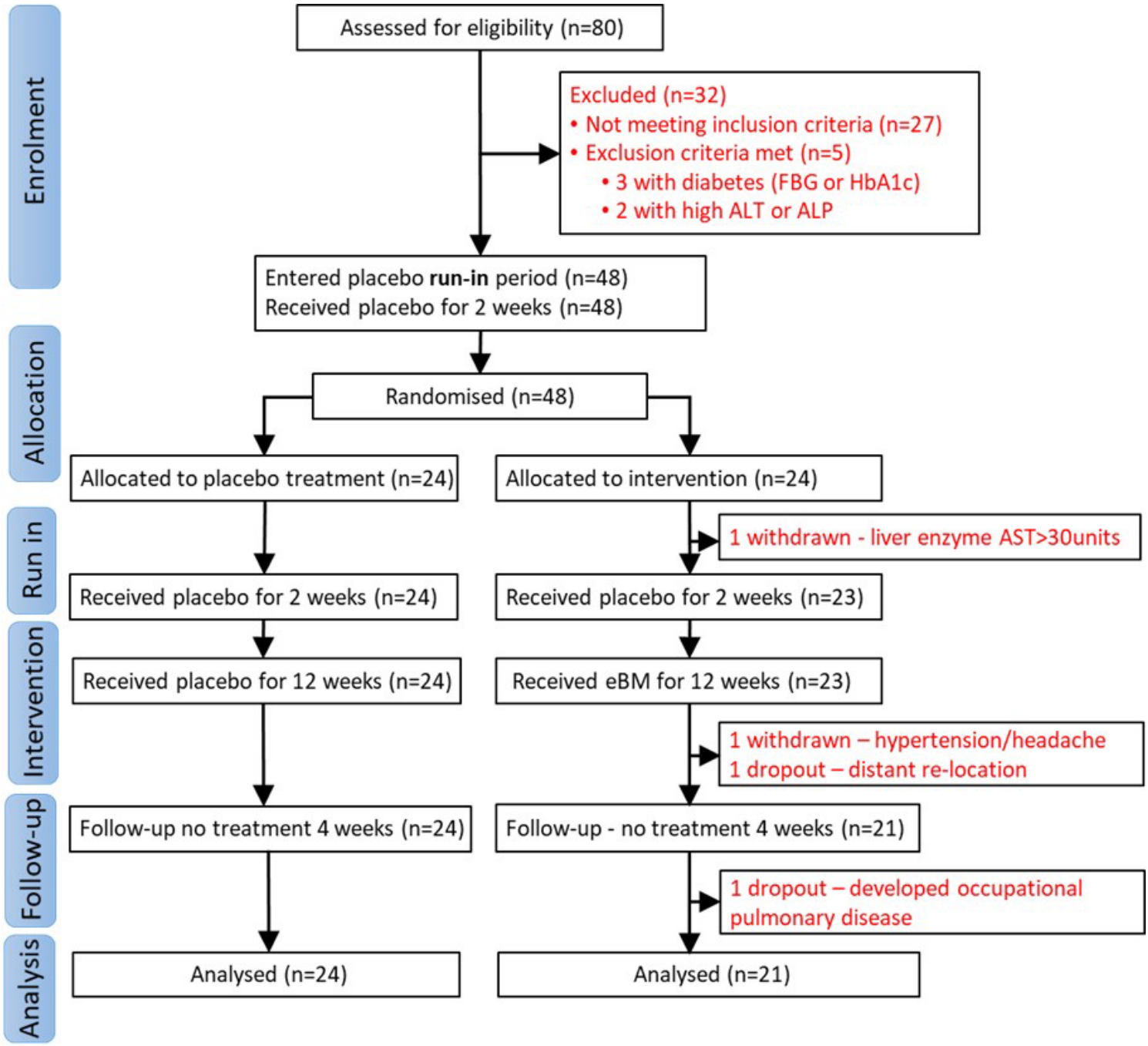

Figure 5

Participant flow diagram.

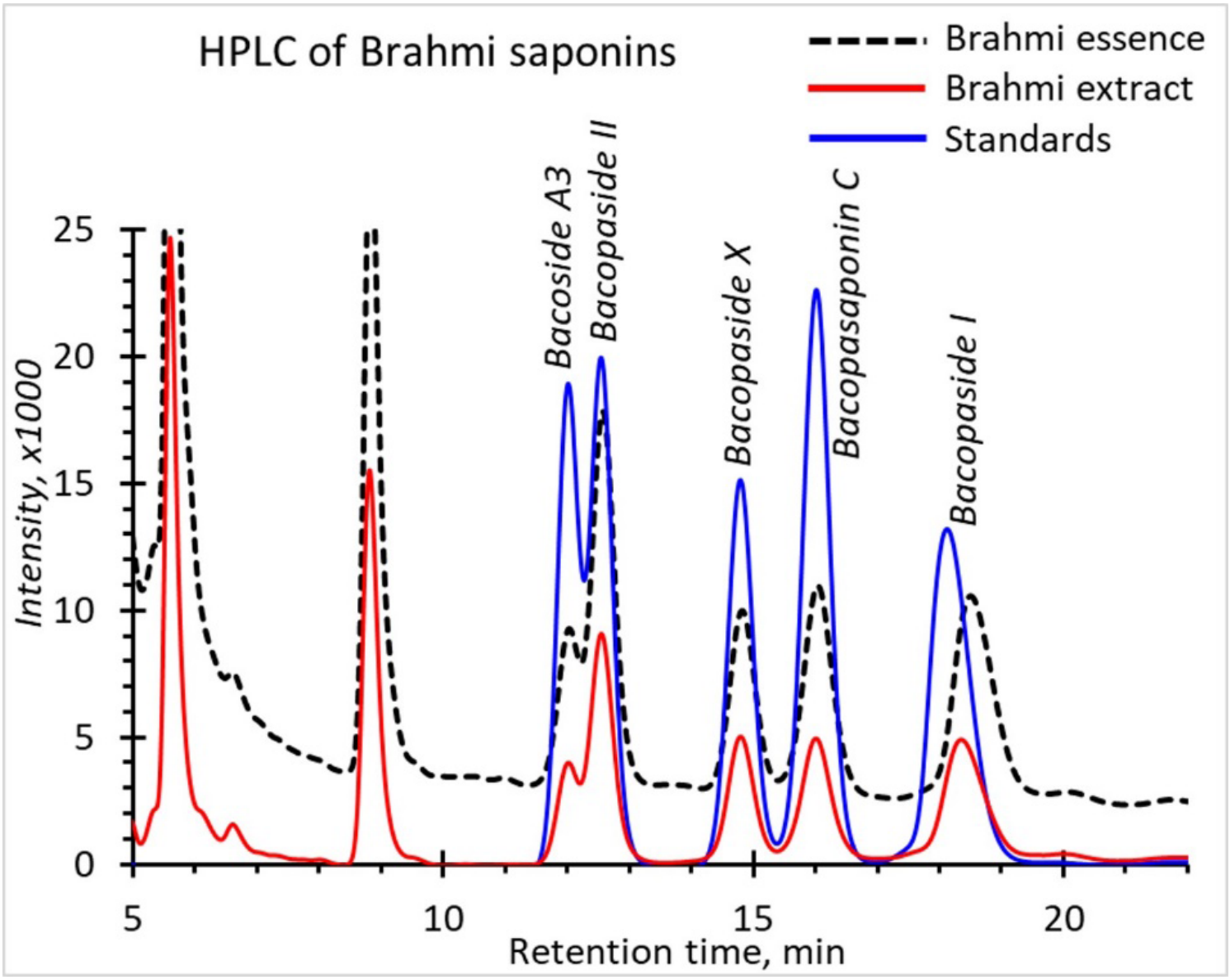

Figure 6

HPLC chromatogram of saponins in the test product, eBM and the standards.

	Run-in		Treatment												Follow up			
Week of trial	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Placebo group, n	48	48	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
eBM group, n			23	23	23	23	23	23	23	23	23	23	23	23	21	21	21	21
Main outcome tests	↑	↑				↑				↑				↑				↑
Querying AEs	↑	↑				↑				↑				↑				↑
Blood test	↑	↑												↑				
Endothelial markers		↑												↑				
Collect intervention	↑	↑				↑				↑								

Figure 7

Trial events, tasks and sequences experienced by participants.

Supplementary Files

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- GraphicalAbstract.tif
- SupplementaryInformationKChootipAmendments2.docx