Journal of Functional Foods 85 (2021) 104671

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

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

Adrian L. Lopresti [a,b,*], Stephen J Smith [a,b], Sinan Ali [c], Alexandra P. Metse [d], John Kalns [e], Peter D. Drummond [b]

[a] Clinical Research Australia, Perth, Western Australia 6023, Australia
[b] Healthy Ageing Research Centre, Murdoch University, Perth, Western Australia 6150, Australia
[c] Salpath Functional Pathology, Sydney, New South Wales 2016, Australia
[d] School of Health and Behavioural Sciences, University of the Sunshine Coast, Sippy Downs, Queensland 4556, Australia
[e] Hyperion Biotechnology, San Antonio, Texas 78216, USA

ARTICLE INFO

Keywords:
Bacopa monnieri
Sleep
Stress
Cortisol
Herbal
Clinical trial

ABSTRACT

In this 28-day, randomised, double-blind, placebo-controlled trial, 100 adults with self-reported poor sleep received either a placebo or a standardised *Bacopa monnieri* extract (150 mg twice daily). Outcome measures included the Bergen Insomnia Scale (primary outcome measure), Functional Outcomes of Sleep Questionnaire, Pittsburgh Sleep Diary, Short Form-36 Health Survey, and the Depression, Anxiety, and Stress Scale. Changes in salivary concentrations of cortisol, dehydroepiandrosterone sulfate, immunoglobulin A (sIgA), α-amylase (sAA), C-reactive protein, melatonin, and the fatigue biomarker index were also assessed. Based on the Bergen Insomnia Scale, *Bacopa monnieri* did not improve sleep patterns more than the placebo; however, it was associated with greater improvements in emotional wellbeing, general health, and pain-related symptoms. *Bacopa monnieri* was also associated with greater reductions in sIgA and sAA compared to the placebo. Future clinical trials using varying doses, treatment periods, and objective outcome measures will be important to validate these findings.

1. Introduction

Common treatments for insomnia include pharmacological interventions, and behavioural and psychological interventions such as cognitive behavioural therapy for insomnia, stimulus control, sleep restriction therapy, relaxation training, and sleep hygiene recommendations (Edinger et al., 2021; Palagini et al., 2020). However, despite their efficacy, a significant portion of individuals obtain no benefit or continue to experience residual insomnia-related symptoms (Davidson, Dickson, & Han, 2019). Pharmacological treatments for insomnia include controlled-release melatonin, tricyclic antidepressants, benzodiazepines, antihistamines, antiepileptics, and atypical antipsychotics (Matheson & Hainer, 2017). These medications have moderate efficacy but are associated with several adverse effects including sedation, weight gain, dizziness, headaches, and gastrointestinal complaints (Fitzgerald & Vietri, 2015; Lie, Tu, Shen, & Wong, 2015). Many sleep-promoting medications, particularly some benzodiazepines, are also associated with problematic withdrawal effects including rebound insomnia (Hintze & Edinger, 2018).

The pathophysiology of insomnia is not fully understood but is believed to be multifaceted. These include genetic variations and disturbances in the hypothalamus-pituitary-adrenal (HPA) axis activity, melatonergic activity, neurotransmitter action (e.g., gamma-aminobutyric acid, noradrenaline, and serotonin), and neural circuitry

Abbreviations: BIS, Bergen Insomnia Scale; BMI, body mass index; CAR, cortisol awakening response; COX-2, cyclooxygenase-2; CRP, C-reactive protein; DASS-21, Depression, Anxiety, and Stress Scale-21; DHEA-S, dehydroepiandrosterone sulfate; ELISA, enzyme-linked immune sorbent assays; FBI, Fatigue biomarker index; FOSQ-10, Functional Outcomes of Sleep Questionnaire; HPA, hypothalamus-pituitaryadrenal; HRP, horse radish peroxidase; IL, interleukin; MOA, monoamine oxidase; OD, optical density; OF, oral fluid; PSD, Pittsburgh Sleep Diary; sAA, salivary α-amylase; SF-36, Short Form-36 Health Survey; sIgA, salivary immunoglobulin A; TMB, tetramethyl benzidine; TNF-α, Tumor necrosis factor-α.

* Corresponding author at: A: 38 Arnisdale Rd Duncraig, WA 6023, Australia.
E-mail address: adrian@clinicalresearch.com.au (A.L. Lopresti).

https://doi.org/10.1016/j.jff.2021.104671
Received 24 March 2021; Received in revised form 1 July 2021; Accepted 2 August 2021
Available online 5 August 2021

(Levenson, Kay, & Buysse, 2015; Pigeon & Cribbet, 2012). There is also increasing research to suggest a bi-directional relationship between inflammation, oxidative stress, and sleep quality (Gulec et al., 2012; Villafuerte et al., 2015). For example, in a representative sample of 319 Swedish women, higher C-reactive protein (CRP) concentrations were positively associated with self-reported sleep disturbances, specifically sleep maintenance and early morning awakenings (Ghilotti et al., 2021). In a study on adults with primary insomnia, the mean blood concentration of the antioxidant enzyme glutathione peroxidase was lower, and concentrations of malondialdehyde, a marker of lipid peroxidation, were higher compared to matched healthy volunteers (Gulec et al., 2012).

Plants, herbs, spices, and their extracts (henceforth referred to as herbs) contain multiple constituents that have been demonstrated in *in vitro*, animal, and human trials to have anti-inflammatory, antioxidant, adaptogenic, analgesic, and neuroprotective effects (Liu et al., 2013; Wink, 2015). *Bacopa monnieri* (also known as Brahmi, water hyssop, and *Herpestis monniera*) is a creeping perennial plant that has been shown to have anticonvulsant, antidepressant, analgesic, anti-inflammatory, anxiolytic, adaptogenic, and neuroprotective effects (Aguiar & Borowski, 2013; P. S. Saha et al., 2020; Sukumaran, Amalraj, & Gopi, 2019). *Bacopa monnieri* also has an important role in cellular homeostasis by modulating apoptosis through autophagy (Das et al., 2016; S. Saha et al., 2020; Smith et al., 2018). As a functional food, *Bacopa monnieri* is often referred to as a nootropic agent that improves memory and mental acuity (Brimson et al., 2021; Sukumaran et al., 2019). In India, it has been used as a component in drinks, biscuits, syrups, jellies, and breakfast cereals (Devendra et al., 2018). The main bioactive constituents of *Bacopa monnieri* believed to be associated with its cognitive effects are saponins called bacosides, with bacosides A and B the most studied constituents (Sukumaran et al., 2019). Specifically relating to the pathophysiological processes associated with sleep disturbances, *Bacopa monnieri* has been shown to influence HPA-axis activity (S. Kumar & Mondal, 2016; Zu et al., 2017), neurotransmitter concentrations of dopamine and serotonin (Rauf et al., 2012; Sheikh et al., 2007), and antioxidant and inflammatory activity (Nemetchek, Stierle, Stierle, & Lurie, 2017; Stough, Singh, & Zangara, 2015). In experimental studies, bacoside A inhibited inflammatory cytokine production (Madhu, T, & S, 2019), reduced free radical damage in the liver and brain (Sekhar, Viswanathan, & Baby, 2019), inhibited inflammatory cytokine production in the brain (Nemetchek et al., 2017), and inhibited beta-amyloid cytotoxicity (Malishev et al., 2017). Due to the association between inflammation, oxidative stress, HPA-axis activity, and neurotransmitter concentrations, *Bacopa monnieri* has promise as a sleep-promoting and mood-enhancing agent. In human trials, *Bacopa monnieri* has been shown to have positive cognitive (Abdul Manap et al., 2019; Hingorani, Patel, & Ebersole, 2012; N. Kumar et al., 2016; Stough et al., 2008) and anxiolytic (Benson et al., 2014; Calabrese et al., 2008) effects; however, there have been no trials examining its effect on sleep. The aim of this human trial was to examine the effects of a *Bacopa monnieri* extract (Bacognize®) on sleep, quality of life, and fatigue in adults with self-reported poor sleep. Changes in several salivary hormones associated with stress, sleep, fatigue, and inflammation were also evaluated to help elucidate the potential mechanisms of action associated with *Bacopa monnieri* supplementation.

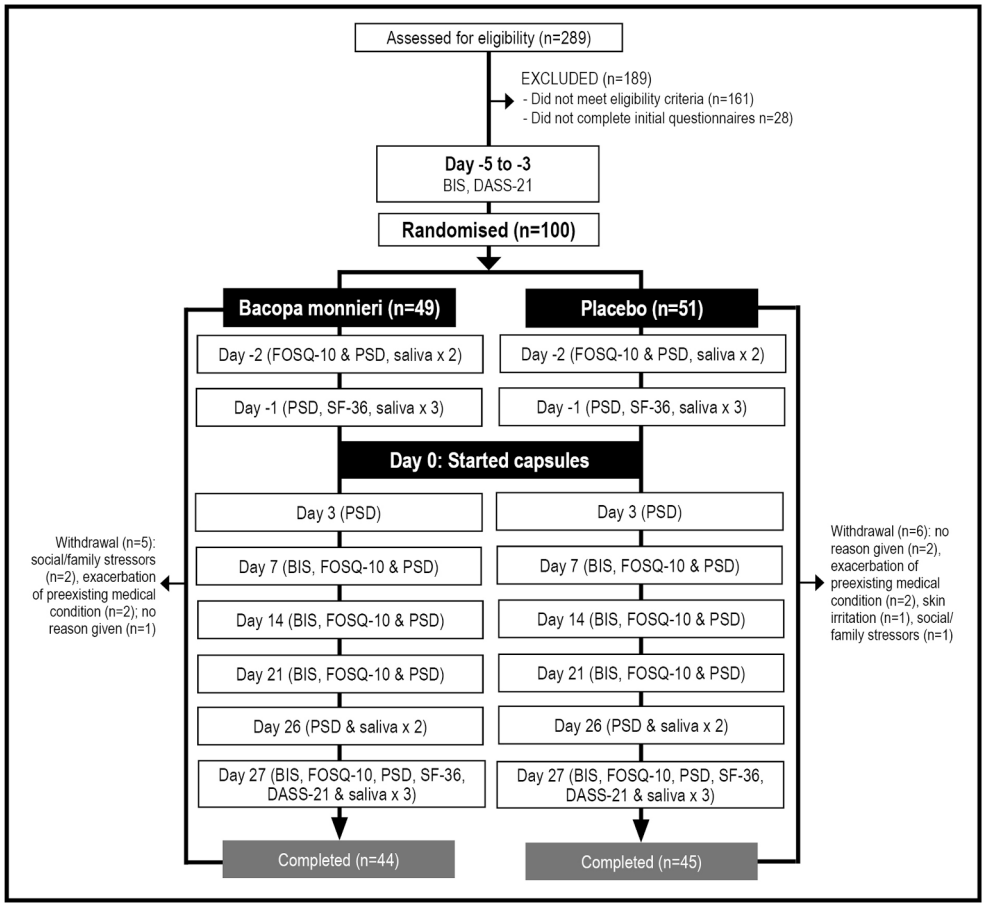

Fig. 1. Systematic Illustration of Study Design. BIS = Bergen Insomnia Scale; DASS-21 = Depression, Anxiety, Stress Scale – 21; FOSQ-10 = Functional Outcomes of Sleep Questionnaire; PSD = Pittsburgh Sleep Diary; SF-36 = Short Form-36.

2. Materials and methods

2.1. Study design

This was a two-arm, parallel-group, 28-day, randomised, double-blind, placebo-controlled trial (Fig. 1). The trial protocol was approved by the Human Research Ethics Committee at the National Institute of Integrative Medicine (approval number 0066E_2020) and was prospectively registered with the Australian New Zealand Clinical Trials Registry (Trial ID. ACTRN12620000770965). Based on a single outcome variable, an *a priori* power analysis was undertaken to estimate the required sample size. Even though there has been no study examining the effects of *Bacopa monnieri* on sleep quality, an effect size of 0.6 was predicted based on a previous trial examining the sleep-enhancing effects of a herbal ingredient (Lopresti, Smith, Metse, & Drummond, 2020). Assuming a power of 80% and a type one error rate (alpha) of 5%, the number of participants required per group to find an effect on the Bergen Insomnia Scale (BIS) was estimated as 36. After allowing for an approximate 20% drop out rate, we aimed to recruit 50 participants per group.

2.2. Recruitment and randomisation

Participants were recruited across Perth, Western Australia through social media advertisements between July and August 2020. Interested participants were directed to a website page that provided information about the trial and a link to complete an online screening questionnaire. This questionnaire screened for mental health symptoms, medication use, history of medical or psychiatric disorders, alcohol, nicotine, and other drug use, supplement and vitamin intake, and pregnancy/breast-feeding status. If assessed as likely eligible, volunteers participated in a telephone interview comprising a structured series of questions to further clarify details pertaining to the eligibility criteria and to obtain further demographic details. Suitable participants were then required to complete online versions of the Bergen Insomnia Scale (BIS), Depression, Anxiety, and Stress Scale (DASS-21), and an informed consent form. Eligible and consenting participants were randomly allocated to one of two groups (*Bacopa monnieri* or placebo) using a randomisation calculator (http://www.randomization.com). The randomisation calculator ensured sequence concealment. The randomisation structure comprised 10 randomly permuted blocks, containing 10 participants per block. The participant identification number was assigned based on the order of participant enrolment in the study. All capsules were packed in identical bottles labelled by two intervention codes (held by the study sponsor until final data collection). Participants and study investigators were blind to treatment group allocation until all outcome data were collected. No financial compensation was provided to participants for volunteering in this study, although at the end of the study, participants allocated to the placebo condition were offered a free 4-week supply of *Bacopa monnieri* capsules.

2.3. Participants

Inclusion criteria: physically-healthy, male and female participants aged 18 to 70 years, with a score of 3 or more on at least one of the first four questions on the BIS and a score of 3 or more on at least one of the last two questions on the BIS were recruited for this trial. All participants were medication-free for at least 4 weeks apart from the contraceptive pill and no more than once per week use of pain-relieving medications. Volunteers had a body mass index (BMI) between 20 and 30 and a usual bedtime between 9 pm and 12 am. Participants were fluent in English and consented (via an online consent form) to all pertinent aspects of the trial.

Exclusion criteria: Participants were ineligible to participate in the study if they were employed in night shift work or rotational shift work. People experiencing a sleep disorder other than moderate insomnia (e.g, sleep apnoea, periodic limb movement disorder, restless legs syndrome), persistent, severe sleep disturbance greater than 1 year, diagnosis of a mental health disorder (other than mild depressive or anxiety symptoms as measured by the Depression, Anxiety and Stress Scale-21), coffee intake greater than 3 cups per day (or equivalent caffeine intake from other caffeinated drinks e.g., tea, energy drinks), and alcohol consumption greater than 14 standard drinks per week were also ineligible for the study. Participants were also ineligible for the study if they reported experiencing external factors that may affect their sleep patterns (e.g., infant/children regularly awakening, excessive noise, snoring partner); were receiving non-pharmacological treatment for sleep disorders (e.g., cognitive behavioural therapy, relaxation therapy); had a current or 12-month history of illicit drug use; were taking supplements that may affect sleep; were taking *Bacopa monnieri* supplements; or had a diagnosed medical condition including but not limited to: diabetes, hyper/hypotension, cardiovascular disease, a gastrointestinal disease requiring regular use of medications, gallbladder disease/gallstones/biliary disease, endocrine disease, psychiatric disorder, neurological disease (Parkinson's, Alzheimer's disease, intracranial haemorrhage, head or brain injury), or acute or chronic pain affecting sleep. Pregnant women, women who were breastfeeding, or women who intended to fall pregnant were also ineligible to participate in the study.

2.4. Interventions

Placebo and *Bacopa monnieri* capsules were identical in appearance, being matched for colour coating, shape, and size. The active treatment, supplied by Verdure Sciences Inc. (Noblesville, Indiana, USA), contained 150 mg of a standardised *Bacopa monnieri* extract (Bacognize®). This dose was chosen as previous studies using this identical extract and dose have demonstrated cognitive-enhancing effects in both younger and older adults (Hingorani et al., 2012; N. Kumar et al., 2016; Stough et al., 2008). Bacognize® is a standardised hydroalcoholic extract of the whole herb *Bacopa monnieri* (L.) Wettst. It is standardised by the United States Pharmacopeial Convention method (using High-performance liquid chromatography) to 12% total Bacopa glycosides (Bacopaside I, Bacoside A3, Bacopaside II, Jujubogenin isomer of Bacopasaponin C, Bacopasaponin C). Each capsule contained 150 mg of Bacognize® extract. The placebo capsules (microcrystalline cellulose powder) contained the same excipients as the active capsules. All participants were instructed to take one capsule, twice daily (morning and evening), with or without food for 27 days. Medication adherence was measured by participants providing a capsule count every 7 days. Efficacy of participant treatment blinding was examined by asking participants to predict group allocation (placebo, bacopa, or uncertain) at the completion of the study. *Bacopa monnieri* and placebo capsules were mailed to participants with directions for use provided on capsule bottles. Participants were also provided with an information sheet about capsule intake and what to do if they missed a dose. This information was also verbally conveyed to participants during their initial telephone interview.

2.5. Outcome measures

2.5.1. Primary outcome measure:

Bergen Insomnia Scale (BIS): The BIS is a validated six-item questionnaire that assesses difficulties with sleep initiation, sleep maintenance, early morning awakening, nonrestorative sleep, daytime impairment, and satisfaction with sleep. The BIS correlates highly with other validated sleep questionnaires such as the Pittsburgh Sleep Quality Index (Pallesen et al., 2008). For each question, respondents indicate how many days per week (0 to 7 days) they experienced each sleep-related problem. A total composite score is calculated by adding together the scores for each item, yielding a total score with a possible range of 0 to 42. The BIS was completed at baseline (from days −5 to −3), day 7, 14, 21, and 27.

2.5.2. Secondary outcome Measures: Questionnaires and diaries

Functional Outcomes of Sleep Questionnaire (FOSQ-10): The FOSQ-10 comprises 10 questions evaluating the respondent's quality of life as it relates to disorders of excessive sleepiness. A total score is calculated plus subscale scores for the five domains of day-to-day life comprising (1) general productivity, (2) activity levels, (3) vigilance, (4) social outcomes, and (5) intimacy and sexual relationships. The FOSQ-10 has good psychometric properties and similar reliability and validity to the longer version of the original FOSQ (Chasens, Ratcliffe, & Weaver, 2009). The FOSQ-10 was completed on day −2, 7, 14, 21, and 27.

Pittsburgh Sleep Diary (PSD): the PSD is a 14-item sleep diary that respondents complete upon awakening. The PSD shows good retest reliability over a mean inter-test interval of 22 months. Scores also correlate with circadian type, subjective sleep quality, and objective actigraphy measurements (Monk et al., 1994). Scores are calculated for total sleep time (hours), sleep latency (minutes), number of awakenings after sleep onset, sleep quality rating [5-point Likert rating ranging from very bad (1) to very good (5)], mood rating at final awakening [5-point Likert rating ranging from very calm (1) to very tense (5)], and alertness rating at final awakening [5-point Likert rating ranging from very sleepy (1) to very alert (5)]. The PSD was completed on day −2, day −1, 3, 7, 14, 21, 27 and 28.

Short Form-36 Health Survey (SF-36): The SF-36 is a self-report measure assessing quality of life. It consists of eight scaled scores measuring (1) vitality, (2) physical functioning, (3) bodily pain, (4) general health perceptions, (5) physical role functioning, (6) emotional role functioning, (7) social role functioning, and (8) emotional well-being. The SF-36 is a commonly-used outcome measure of quality of life with strong psychometric properties (McHorney, Ware, & Raczek, 1993; Ware & Sherbourne, 1992). Scoring for the SF-36 was based on the algorithm developed by RAND Health Care where high scores indicate a more favourable health state (Hays, Sherbourne, & Mazel, 1993). Based on previous factor analyses, the SF-36 has been identified as having two factors: a mental component consisting of the social functioning, emotional wellbeing, role limitations due to emotional problems, and vitality subscale scores; and a physical component consisting of the physical function, general health, pain, and role limitations due to physical health subscale scores (Ware et al., 1995). The SF-36 was completed on days −1 and day 27.

Depression, Anxiety, and Stress Scale – 21 (DASS-21): The DASS-21 is a validated self-report measure assessing symptoms of stress, anxiety, and depression (Brown, Chorpita, Korotitsch, & Barlow, 1997). Twenty-one questions are rated on a 4-point scale (0–3), ranging from never to almost always (lower scores indicate a reduction in symptoms). Subscale scores for depression, anxiety, and stress are calculated. The DASS-21 was completed at baseline (days −5 to −3) and on day 27.

2.5.3. Secondary outcome Measures: Salivary hormones

Salivary cortisol (morning and evening). Cortisol provides a measure of HPA axis activity. Concentrations are altered during times of stress, and differences (increased and decreased concentrations) have been observed in adults with insomnia compared to adults with healthy sleep (Nicolaides, Vgontzas, Kritikou, & Chrousos, 2000).

Dehydroepiandrosterone sulfate (DHEA-S). DHEA-S is an endogenous steroid that is produced by the adrenal cortex. Concentrations of DHEA-S are altered during times of stress (Walker, Pfingst, Carnevali, Sgoifo, & Nalivaiko, 2017) and are higher in adults with post-traumatic stress disorder and trauma-exposed adults (van Zuiden et al., 2017).

Salivary immunoglobulin A (sIgA). sIgA has important immunological functions and is altered after exposure to various psychosocial stressors (Brandtzaeg, 2013; Tsujita & Morimoto, 1999; Valdimarsdottir & Stone, 1997).

Salivary α-amylase (sAA). sAA is a marker of stress and autonomic nervous system activity. Concentrations are lowered after mind–body interventions such as stress-reduction programs, self-compassion training, and mindfulness-based interventions (Ali & Nater, 2020; Arch et al., 2014; Duchemin, Steinberg, Marks, Vanover, & Klatt, 2015; Limm et al., 2011).

Salivary C-reactive protein (CRP). CRP is an acute-phase inflammatory protein that increases in response to injury, infection, and inflammation (Sproston & Ashworth, 2018). CRP is typically measured in blood although salivary concentrations have been shown to correlate modestly with serum concentrations (Out, Hall, Granger, Page, & Woods, 2012; Pay & Shaw, 2019). Concentrations of CRP are higher in people with insomnia and sleep disturbances (Ghilotti et al., 2021; Meier-Ewert et al., 2004).

Salivary melatonin. Melatonin is a hormone primarily released by the pineal gland and has an important role in the regulation of the sleep-wake cycle (Low, Choo, & Tan, 2020). Salivary melatonin concentrations are altered during times of stress and have been found, albeit inconsistently, to be associated with sleep quality (Ito et al., 2013; Kennaway, 2020).

Fatigue biomarker index (FBI). The FBI is described as an objective fatigue measure based on the ratio in concentrations of two salivary peptide fragments. The FBI has been shown to correlate with evaluations of perceived exertion in male cyclists (Michael, Daugherty, Santos, Ruby, & Kalns, 2012), predicted success or failure in military training candidates (Kalns et al., 2011), and was altered after 48 hrs of sleep deprivation (Michael, Valle, Cox, Kalns, & Fogt, 2013). Lower FBI reflects greater fatigue.

Adverse events: Tolerability and safety of supplement intake by participants were assessed every 7 days via an online question querying adverse effects that were believed to be associated with supplement intake. Participants were also requested to contact the researchers immediately if any adverse effects were experienced.

2.5.4. Data collection procedures

Initial screening questionnaires comprising the BIS and DASS-21 were completed online. A response booklet containing copies of the required questionnaires and sleep diaries was then mailed to all participants. The dates for completion of each questionnaire, diary, and saliva collection were recorded in the booklet. Participants were also advised to keep their response booklet near their bed and to complete it within 30 min after awakening.

To measure salivary hormones, participants were provided with small collection tubes and the whole saliva was collected by unstimulated passive drool. These samples were collected in participants' homes on days −2, −1, 26 and 27. There were exactly 28 days between saliva collections to ensure pre- and post-saliva collections occurred on the same day of the week. Salivary testing procedures used in this study are detailed in supplementary file 1 and saliva collection details are as follows:

(1) On days −2 and 26 (morning collection) before brushing their teeth and consuming any food or drink, participants were instructed to collect approximately 5 mls of saliva 30 min after waking. To ensure good saliva flow, participants were permitted to drink a glass of water no sooner than 15 min prior to their saliva collection. This sample was used to measure cortisol, DHEA-S, sIgA, sAA, and C-reactive protein (CRP).
(2) On days −2 and 26 (evening collection) participants were instructed to collect approximately 5 mls of saliva at 10 pm. They were requested to not consume any food or drink at least 15 min before collecting this sample. If participants went to bed earlier than this time, they were requested to collect the sample before going to bed. However, they were requested to collect the days −2 and 26 evening saliva samples at the same time. This sample was used to measure cortisol and melatonin.
(3) On days −1 and 27, participants were requested to collect a saliva sample three times throughout the day, upon waking, at midday, and in the evening. Participants were instructed to collect this sample at least 15 min away from consuming any food or drink.

Samples were collected by chewing on a cotton sponge for 2 min. This sample was used to measure the FBI.

2.6. Statistical analysis

An independent samples *T*-test or Mann-Whitney *U* test was used to compare demographic variables across the two treatment groups for continuous variables, and Pearson's Chi-square was used to compare categorical data. To assess for the effects of *Bacopa monnieri*, compared to placebo, on primary and secondary outcomes (BIS and FOSQ-10; PSD; SF-36 physical component; SF-36 mental component; and salivary hormones), multivariate, repeated-measures analyses of variance (ANOVA) were computed. If the time × group (*Bacopa monnieri* versus placebo) interaction was significant, further univariate repeated measures ANOVA analyses were conducted to examine within-group changes over time and group × time interaction effects for relevant subscale scores and hormonal measurements. Normality of data was assessed by the visual inspection of Q-Q plots and an analysis of skewness and kurtosis. This indicated that the questionnaire and diary data were mostly normally distributed. However, salivary hormone concentrations were not normally distributed so logarithmic transformations were conducted, which resulted in normalisation of data. FBI is a log-transformed measure and was thus evaluated without transformative steps. Where necessary, degrees of freedom in univariate analyses were adjusted using the Greenhouse-Geisser approach to correct for violations of the sphericity assumption. The BIS total score was analysed for changes from baseline (day −5 to −3), days 7, 14, 21, and 27; the FOSQ-10 total score from days −2, 7, 14, 21, and 27; the DASS-21 from baseline (day −5 to −3) to day 27; the PSD scores from mean baseline (days −2 and −1) to mean follow-up (mean score across days 3, 7, 14, 21, 26, and 27); salivary hormone concentrations from baseline to day 26; and mean FBI concentrations (morning, midday, and evening collections) from baseline to day 27. Data from all participants who returned their response booklets were included in analyses. All data were analysed using SPSS (version 26; IBM, Armonk, NY). The critical *p*-value was set at $p \leq 0.05$ for all analyses.

3. Results

3.1. Study population

3.1.1. Baseline questionnaire and demographic information

As detailed in Fig. 1, from 289 people who completed the initial online screening questionnaire, 189 individuals did not complete the initial baseline questionnaires (n = 28) or were ineligible (n = 161). One-hundred volunteers participated in the study and questionnaire/diary data from 89 participants who returned questionnaire booklets were used for statistical analyses. Pre and post salivary hormone samples (cortisol, DHEA-S, melatonin, CRP, sIgA, and sAA) were obtained from 87 participants. Full pre and post-FBI samples (morning, midday, and evening) were obtained from 78 participants. Details of participant demographic information and baseline scores of the total recruited sample are detailed in Tables 1 and 2. Eleven participants withdrew or did not return response booklets. There were no significant differences in dropout rates across groups. Reasons for withdrawal included exacerbation of a pre-existing medical condition (n = 4), social/family stressors (n = 3) no reason given (n = 2), and skin irritation (n = 1).

3.2. Outcome measures

3.2.1. BIS and FOSQ-10

Change in the BIS and FOSQ-10 scores across the two treatment groups and repeated measures ANOVA significance levels are detailed in Table 3. BIS and FOSQ-10 scores decreased over time ($F_{8,80} = 20.47$, $p < .001$), comprising a reduction from baseline to day 27 in BIS scores of 50% and 47% in the *Bacopa monnieri* and placebo groups, respectively

Table 1
Baseline Demographic Details of Participants.

		Bacopa n = 49	Placebo n = 51	p-value
Age	Mean	49.02	51.02	0.359
	SE	1.56	1.50	[a]
Sex	Female (n)	39	38	0.546
	Male (n)	10	13	[a]
BMI	Mean	25.56	26.03	0.508
	SE	0.49	0.51	[a]
Marital status	Single (n)	17	11	.144[b]
	Married/defacto (n)	32	40	
Educational level	Secondary (n)	24	17	.137[b]
	Tertiary (n)	13	23	
	Post-graduate (n)	12	11	
Exercise level (n)	Never/rarely (n)	4	11	.180[b]
	1 to 2 times a week (n)	3	3	
	3 to 5 times a week (n)	19	12	
	6 + times a week (n)	23	25	
Duration of sleep problems	<6 months (n)	5	3	.472[b]
	6 to 12 months (n)	10	6	
	1 to 2 years (n)	7	8	
	2 to 5 years (n)	10	10	
	5 to 10 years (n)	3	9	
	10 or more years (n)	14	15	
BIS	Mean	27.94	28.80	0.576
	SE	1.14	1.04	[a]
FOSQ-10	Mean	30.70	30.11	0.573
	SE	0.82	0.66	[a]
DASS Depression	Mean	4.65	5.49	0.416
	SE	0.64	0.80	[a]
DASS Anxiety	Mean	3.43	4.35	0.313
	SE	0.54	0.73	[a]
DASS Stress	Mean	11.55	9.80	0.237
	SE	1.10	0.98	[a]
DASS Total	Mean	19.63	19.65	0.996
	SE	1.96	2.16	[a]
		n = 44	n = 45	
SF-36 Physical Functioning	Mean	88.98	89.44	0.872
	SE	2.26	1.82	[a]
SF-36 Role limitations due to physical health	Mean	82.23	84.64	0.509
	SE	2.45	2.69	[a]
SF-36 Role limitations due to emotional problems	Mean	82.75	82.04	0.860
	SE	2.72	2.90	[a]
SF-36 Vitality	Mean	49.77	51.56	0.604
	SE	2.40	2.44	[a]
SF-36 Emotional well-being	Mean	69.32	71.78	0.437
	SE	2.20	2.25	[a]
SF-36 Social functioning	Mean	78.55	77.69	0.837
	SE	2.63	3.20	[a]
SF-36 Pain	Mean	72.61	75.38	0.459
	SE	2.45	2.79	[a]
SF-36 General Health	Mean	67.61	69.33	0.609
	SE	2.52	2.22	[a]
PSD Time to sleep onset (min)	Mean	30.32	30.92	0.926
	SE	4.41	4.71	[a]
PSD Total sleep time (min)	Mean	419.39	414.96	0.798
	SE	11.95	12.48	[a]
PSD Number of waking	Mean	3.13	3.68	0.651
	SE	0.34	1.16	[a]
PSD Sleep Quality Rating	Mean	2.86	2.91	0.743
	SE	0.10	0.10	[a]
PSD Mood rating after waking	Mean	2.81	2.64	0.329
	SE	0.11	0.12	[a]
PSD Alertness rating after waking	Mean	2.72	2.74	0.869
	SE	0.12	0.13	[a]

a = Independent samples *t*-test; b = Chi-square Test; BIS = Bergen Insomnia Scale; BMI = Body Mass Index; SE = standard error; DASS-21 = Depression, Anxiety, and Stress Scale − 21; FOSQ-10 = Functional Outcomes of Sleep Questionnaire; PSD = Pittsburgh Sleep Diary; SF-36 = Short-Form-36.

Table 2
Baseline Salivary Hormone Concentrations of Participants.

		Bacopa (n = 42)	Placebo (n = 45)	p-value *
CRP (pg/mL)	Mean	218.92	138.17	0.143
	SE	52.67	28.65	
Cortisol, morning (ug/dL)	Mean	0.40	0.46	0.111
	SE	0.04	0.04	
Cortisol, evening (ug/dL)	Mean	0.07	0.13	0.849
	SE	0.00	0.05	
sIgA (ug/mL)	Mean	302.42	215.35	0.065
	SE	59.10	77.51	
sAA (U/mL)	Mean	75.53	62.32	0.282
	SE	7.02	4.12	
DHEA-S (pg/mL)	Mean	4588.20	3275.91	0.745
	SE	705.99	276.31	
Melatonin (pg/mL)	Mean	7.87	7.12	0.550
	SE	1.10	1.10	
FBI (morning)	Mean	−1.46	−1.61	0.136
	SE	0.076	0.061	
	n	43	44	
FBI (midday)	Mean	−1.95	−2.04	0.200
	SE	0.053	0.044	
	n	42	43	
FBI (evening)	Mean	−2.00	−2.04	0.604
	SE	0.061	0.062	
	N	43	45	

* Independent-samples *t*-test (log10 scores)

(Fig. 2), and an increase in FOSQ-10 scores by 5 and 4% in the *Bacopa monnieri* and placebo groups, respectively. However, changes were similar in both groups; specifically, the between-group main effect for BIS and FOSQ-10 scores ($F_{2,86} = 0.023$, $p = .978$) and the group × time interaction ($F_{8,80} = 0.539$, $p = .823$) were not significant.

3.2.2. Psd

Changes in the PSD scores across the two treatment groups and repeated measures ANOVA significance levels are detailed in Table 4. There was an overall significant change in PSD scores over time ($F_{6,82} = 5.15$, $p < .001$) in both groups. However, changes were similar in both groups; specifically, the between-group main effect for PSD scores ($F_{2,82} = 0.573$, $p = .751$) and the group × time interaction ($F_{6,82} = 0.958$, $p = .459$) were not significant. Graphical illustrations of changes in PSD scores are detailed in supplementary file 1, figure S1.

3.2.3. Sf-36

Changes in the SF-36 scores across the two treatment groups and repeated measures ANOVA significance levels are detailed in Table 5. The multivariate analysis revealed there was an overall significant increase in both physical ($F_{4,84} = 8.25$, $p < .001$) and mental component ($F_{4,84} = 13.21$, $p < .001$) scores over time in both groups. There was no significant between-group main effect for SF-36 physical ($F_{4,84} = 0.481$, $p = .750$) or mental component scores ($F_{4,84} = 0.607$, $p = .659$); however, there were statistically-significant group × time interactions for both physical ($F_{4,84} = 3.10$, $p = .020$) and mental component scores ($F_{4,84} = 2.59$, $p = .043$). On the mental component subscale scores, univariate analyses revealed that there was a statistically-significant group × time interaction for the emotional well-being score ($F_{1,87} = 5.99$, $p = .016$). On the physical component subscale scores, univariate analyses revealed there were statistically-significant group × time interactions for pain ($F_{1,87} = 6.69$, $p = .011$) and general health ($F_{1,87} = 5.07$, $p = .027$) subscores. Compared to baseline, supplementation with *Bacopa monnieri* was associated with a 14% improvement in emotional well-being ($F_{1,43} = 22.34$, $p = <0.001$), 12% improvement in general - health ($F_{1,43} = 16.85$, $p < .001$), and 16% reduction in pain ($F_{1,43} = 20.76$, $p < .001$). In contrast, the placebo was associated with a 6% improvement in emotional well-being ($F_{1,44} = 8.10$, $p = .007$), 4% improvement in general health ($F_{1,44} = 4.52$, $p = .039$), and a non-significant 3% reduction in pain ($F_{1,44} = 0.52$, $p = .474$). Graphical illustrations of changes in PSD scores are detailed in supplementary file 1, figure S2.

3.2.4. Dass-21

Changes in the DASS-21 scores across the two treatment groups and repeated measures ANOVA significance levels are detailed in Table 3. There was no overall significant change in DASS-21 scores over time

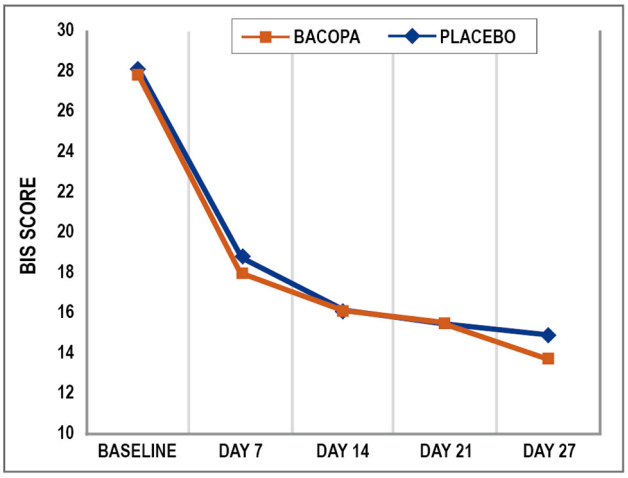

Fig. 2. Change in BIS Scores.

Table 3
Change in Questionnaire Outcome Measures.

		Bacopa (n = 44)					Placebo (n = 45)					Multivariate analysis		
												Time effects*	Group Main effects*	Time × group interaction*
		Baseline	Day 7	Day 14	Day 21	Day 27	Baseline	Day 7	Day 14	Day 21	Day 27			
BIS	Mean	27.77	18	16.25	15.48	13.89	28.18	18.82	16.16	15.48	14.98	<0.001	0.978	0.823
	SE	1.20	1.35	1.34	1.54	1.41	1.12	1.26	1.23	1.27	1.36			
FOSQ-10	Mean	30.70	30.82	31.02	31.45	32.14	30.11	30.47	31.29	31.64	32.18			
	SE	0.82	0.95	0.89	0.87	0.88	0.66	0.70	0.68	0.69	0.72			
DASS-21	Mean	4.64				5.05	5.69				6.04	0.394	0.041	0.885
Depression	SE	0.646				0.782	0.885				0.891			
DASS-21	Mean	3.50				4.05	4.31				4.36			
Anxiety	SE	0.582				0.787	0.804				0.863			
DASS-21	Mean	11.77				10.73	9.82				9.38			
Stress	SE	1.138				1.434	1.063				1.199			

* p-values, repeated measures ANOVA multivariate analysis

Table 4
Change in PSD scores.

		Bacopa (n = 44)			Placebo (n = 45)			Multivariate analysis		
		Mean Baseline	Mean Days 3 to 27	p-value[a]	Mean Baseline	Mean Days 3 to 27	p-value[a]	Time effect[c]	Group main effect[c]	Time × group interaction[c]
Time to sleep onset (min)	Mean	30.32	30.98	0.857	30.92	28.75	0.520	<0.001	0.751	0.459
	SE	4.41	3.87		4.71	3.92				
Total sleep time (min)	Mean	419.39	405.95	0.312	414.96	407.07	0.501			
	SE	11.95	14.31		12.48	9.26				
Number of wakings	Mean	3.13	2.30	<0.001	3.68	1.94	0.126			
	SE	0.34	0.20		1.16	0.15				
Sleep Quality Rating	Mean	2.86	3.11	0.039	2.91	3.15	0.045			
	SE	0.10	0.10		0.10	0.09				
Mood rating after waking	Mean	2.81	2.76	0.620	2.64	2.59	0.552			
	SE	0.11	0.12		0.12	0.10				
Alertness rating after waking	Mean	2.72	3.22	<0.001	2.74	2.90	0.202			
	SE	0.12	0.10		0.13	0.08				

a = within-group, repeated measures ANOVA univariate analysis; b = between-group, repeated measures ANOVA univariate analysis; c = repeated measures ANOVA multivariate analysis

Table 5
Change in SF-36 scores.

		Bacopa (n = 44)			Placebo (n = 45)			Time × group interaction[b]	Multivariate analysis		
		Baseline	Day 27	p-value[a]	Baseline	Day 27	p-value[a]		Time effect[c]	Group Main effect[c]	Time × group interaction[c]
Physical Component											
SF-36 Physical functioning	Mean	88.98	89.77	0.667	89.44	90.67	0.288	0.843	<0.001	0.750	0.020
	SE	2.26	2.20		1.82	1.49					
SF-36 Role limitations due to physical health	Mean	82.23	89.05	0.015	84.64	90.36	0.036	0.770			
	SE	2.45	2.39		2.69	2.25					
SF-36 Pain	Mean	72.61	83.91	<0.001	75.38	77.29	0.474	0.011			
	SE	2.45	2.31		2.79	2.53					
SF-36 General health	Mean	67.61	75.68	<0.001	69.33	72.11	0.039	0.027			
	SE	2.52	2.48		2.22	2.41					
Mental Component											
SF-36 Emotional well-being	Mean	69.32	79.20	<0.001	71.78	75.67	0.007	0.016	<0.001	0.659	0.043
	SE	2.20	1.87		2.25	2.36					
SF-36 Role limitations due to emotional problems	Mean	82.75	90.16	0.004	82.04	89.67	0.001	0.948			
	SE	2.72	2.18		2.90	2.51					
SF-36 Social functioning	Mean	78.55	88.30	<0.001	77.69	82.91	0.076	0.235			
	SE	2.63	2.23		3.20	3.35					
SF-36 Vitality	Mean	49.77	56.91	0.001	51.56	59.93	<0.001	0.635			
	SE	2.40	2.87		2.44	2.32					

a = within-group, repeated measures ANOVA univariate analysis; b = between-group, repeated measures ANOVA univariate analysis; c = repeated measures ANOVA multivariate analysis

($F_{3,85} = 1.00$, $p = .394$) in either group (group × time interaction $F_{3,85} = 0.217$, $p = .885$), but there was a significant between-group main effect for DASS-21 scores ($F_{3,85} = 2.88$, $p = .041$). However, univariate analyses revealed that there was no significant group main effect for the individual subscale scores of depression ($F_{1,87} = 1.14$, $p = .288$), anxiety ($F_{1,87} = 0.385$, $p = .537$), or stress ($F_{1,87} = 1.14$, $p = .289$).

3.2.5. Salivary hormones

Changes in the salivary hormone concentrations across the two treatment groups and repeated measures ANOVA significance levels (based on logarithmic transformations) are detailed in supplementary file 1, table S1. The multivariate analysis revealed there was no overall significant change in hormone concentrations over time ($F_{7,79} = 1.35$, $p = .238$) and no significant between-group main effect ($F_{7,79} = 0.30$, $p = .950$); however, there was a statistically-significant group × time interaction ($F_{7,79} = 2.97$, $p = .008$). Univariate analyses revealed that there were statistically-significant group × time interactions for morning cortisol ($F_{1,85} = 5.58$, $p = .020$), sIgA ($F_{1,85} = 4.99$, $p = .028$), and sAA ($F_{1,85} = 7.23$, $p = .009$) concentrations. Within-group analysis revealed that in the *Bacopa monnieri* group, there were statistically-significant decreases in sIgA ($F_{1,41} = 4.34$, $p = .043$) and sAA ($F_{1,41} = 5.09$, $p = .030$) concentrations over time. In the placebo group, there was a statistically-significant decrease in morning cortisol concentration over time ($F_{1,44} = 6.39$, $p = .015$). Percentage change in hormone concentrations from baseline to day 26 are illustrated in Fig. 3.

3.2.6. Fatigue biomarker index (FBI)

Changes in the salivary FBI concentrations across the two treatment groups and repeated measures ANOVA significance levels are detailed in supplementary file 1, table S2. The multivariate analysis revealed there was no overall statistically significant changes in overall FBI concentrations over time ($F_{3,74} = 0.84$, $p = .474$), between-group main effect

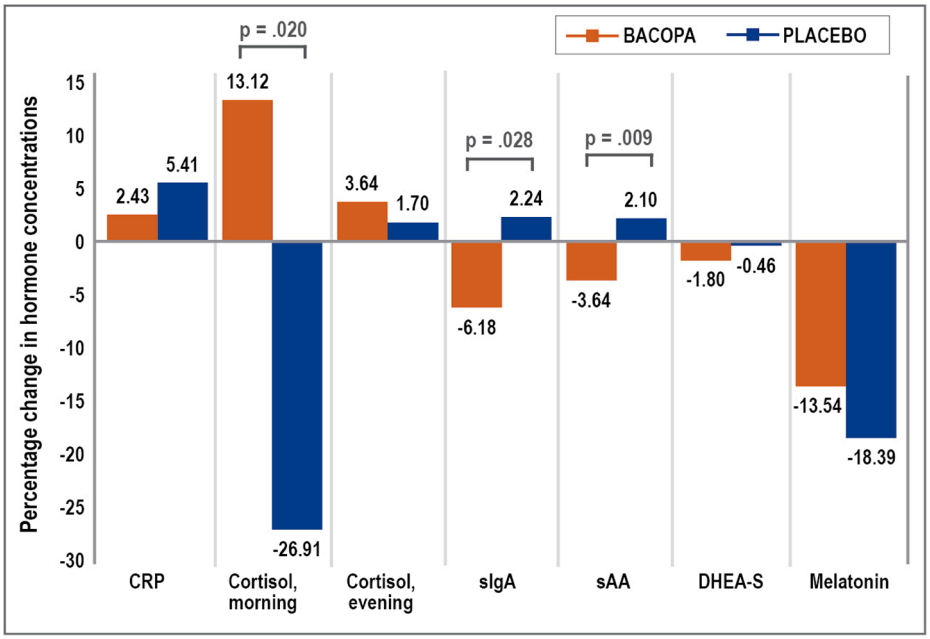

Fig. 3. Percentage Change in Salivary Hormones.

($F_{3,74} = 1.39$, p = .250), or group × time interaction ($F_{3,74} = 0.39$, p = .758).

3.2.7. *Intake of supplements*

On day 7, 14, 21, and 27 participants recorded their quantity of remaining supplements. On day 28, 97% of participants reported taking greater than 80% of their capsules.

3.2.8. *Efficacy of participant blinding*

To evaluate the efficacy of condition concealment over the study, participants were asked at the completion of the study to predict condition allocation (i.e. placebo, *Bacopa monnieri*, or uncertain). Efficacy of group concealment was high as 65% of participants either incorrectly guessed treatment allocation or were unsure.

3.2.9. *Adverse events*

The frequency of self-reported adverse effects is detailed in supplementary file 1, table S3. There were no significant differences in the reports of adverse effects between the groups and no significant adverse events were reported by participants. One participant in the placebo group withdrew from the study due to reports of increased skin itching.

4. Discussion

In this 28-day, randomised, double-blind, placebo-controlled study, supplementation with 150 mg, twice daily of a *Bacopa monnieri* extract was not associated with greater improvements in sleep patterns compared to the placebo. Changes in sleep quality in adults with self-reported sleep disturbances improved significantly in all participants, with reductions in the BIS total score (primary outcome measure) of 50% and 47% in the *Bacopa monnieri* and placebo groups, respectively. Changes in sleep as measured by the PSD and FOSQ-10 also revealed mostly similar improvements over time. However, based on results from the SF-36, *Bacopa monnieri* supplementation was associated with greater improvements in both physical and emotional component scores. Measurements of changes in salivary hormones revealed *Bacopa monnieri* was associated with statistically-significant greater decreases in salivary concentrations of sIgA and sAA. Moreover, there were significant between-group differences in changes in morning cortisol concentrations over the 28 days as demonstrated by decreases in the placebo group and a trend toward increased concentrations in the *Bacopa monnieri* group.

These results suggest that *Bacopa monnieri* supplementation for 28 days at a dose of 150 mg twice daily did not improve sleep patterns more than the placebo in adults with self-reported sleep problems. However, whether intake for a longer duration, higher dose, or different dosage regimen (e.g., once versus twice-daily administration) may result in greater treatment efficacy requires investigation in future trials. Moreover, identical doses were provided to all participants irrespective of age, sex, or weight. In future trials, modifying doses based on these characteristics, particularly weight, will be important to investigate. The high placebo effect in this trial, as demonstrated by an almost 50% reduction in the BIS score, requires consideration as such efficacy is not typical in placebo-controlled trials for insomnia (Perlis, McCall, Jungquist, Pigeon, & Matteson, 2005). Reasons for this high placebo effect could not be determined but may be associated with the easing in work and social restrictions imposed due to the COVID-19 pandemic in Australia during the trial.

Even though further investigation is required to validate the secondary and exploratory outcome findings, *Bacopa monnieri* was associated with greater improvements in emotional wellbeing, pain, and general health compared to the placebo. Although promising, such outcomes require confirmation in future trials utilising validated measures with specifically-recruited populations. In this trial, an improvement in emotional wellbeing was identified but there were no changes in the DASS-21 subscale scores, which is a self-report measure assessing depressive, anxiety, and stress-related symptoms. However, the lack of change in DASS-21 scores may be due to floor effects as, at baseline, participants reported non-clinical levels of depression, anxiety, and stress. In relation to previous investigations into the mood-enhancing effects of *Bacopa monnieri* as an adjunct to an antidepressant medication, *Bacopa monnieri* was associated with improvements in mood in adults with anhedonia (Micheli et al., 2020). Acute, mood-enhancing effects were also identified in another trial on healthy adults exposed to a computerised multitasking activity (Benson et al., 2014). Results from several animal trials have also suggested *Bacopa monnieri* may have anxiolytic or antidepressant effects (Hazra, Kumar, Saha, & Mondal, 2017; Zu et al., 2017). *Bacopa monnieri* contains various metabolites such as saponins, alkaloids and sterols. The main active constituents of *Bacopa monnieri* are saponins known as bacosides with bacoside A the

most studied triterpenoid saponin. Bacoside A is a mixture of four saponins comprising bacoside A3, bacopaside II, jujubogenin isomer of bacopasaponin C (bacopaside X), and bacopasaponin C (Sukumaran et al., 2019). The mood-enhancing effects of *Bacopa monnieri* may be associated with its influence on neurotransmitter availability. For example, tryptophan hydroxylase, an enzyme involved in serotonin synthesis was upregulated in the hippocampus of postnatal rats after the oral treatment of a *Bacopa monnieri* leaf extract containing bacosides (Charles, Ambigapathy, Geraldine, Akbarsha, & Rajan, 2011). An in silico model suggested bacoside A3 may be particularly important in tryptophan hydroxylase function and thus serotonin synthesis (Rajathei, Preethi, Singh, & Rajan, 2014). The *Bacopa monnieri* constituents, bacopaside I and bacoside A have also been shown to inhibit monoamine oxidase activity (MAO-A and MAO-B). These are enzymes involved in the degradation of amine neurotransmitters such as noradrenaline, adrenaline, serotonin, and dopamine (Singh, Ramakrishna, Bhateria, & Bhatta, 2014).

The increase in the pain subscale score of the SF-36 (indicating reductions in pain symptoms) is a unique finding which has not been investigated in any previous human trial. However pain-relieving effects of *Bacopa monnieri* have been identified in animal and *in vitro* trials (Rauf et al., 2013; Shahid, Subhan, Ahmad, & Ullah, 2017; Taznin, Mukti, & Rahmatullah, 2015). Bacopa's analgesic effects may be via its effects on cyclooxygenase-2 (COX-2) activity and the adrenergic, serotonergic, and opioidergic systems (Bhaskar & Jagtap, 2011; Rauf et al., 2013). Bacoside-A also possesses anti-inflammatory actions and in an acute and chronic animal model, downregulated the inflammatory cytokines (interleukins-6 and 17a, and tumour necrosis factor-α) and the inflammatory chemokine CCL-5 (Madhu et al., 2019). In an *in vitro* model, pretreatment with bacoside-A3 before β-amyloid stimulation suppressed the generation of reactive oxygen species, prostaglandin E2 secretion, and the over-expression of COX-2 (Bai & Zhao, 2021). Further confirmation of the pain-relieving effects of *Bacopa monnieri* in populations experiencing pain-related difficulties will be required in future trials.

To help understand the physiological mechanisms associated with *Bacopa monnieri* administration, salivary hormone (morning and evening cortisol, evening melatonin, morning sIgA, sAA, DHEA-S) and FBI concentrations (morning, midday, and evening) were measured. Compared to the placebo, *Bacopa monnieri* was associated with greater reductions in morning sIgA and sAA, and increases in morning cortisol relative to decreased concentrations in the placebo group. sIgA has important immunological functions, including assisting in the prevention of bacteria from forming colonies on mucosal surfaces, and neutralising toxins and enzymes produced by bacteria (Brandtzaeg, 2013; Tsujita & Morimoto, 1999). However, various psychosocial stressors such as academic examination, daily hassles, negative mood, unfavourable daily events, and work demands can influence sIgA concentrations (Tsujita & Morimoto, 1999; Valdimarsdottir & Stone, 1997). The direction of the effects of these stressors on sIgA concentrations is inconsistent as it is influenced by the type of stressor, and the intensity and duration of the stress. For example, sIgA concentrations decreased when measured several days or weeks after academic stress but increased immediately after academic stress (Jemmott & Magloire, 1988; Otsuki et al., 2004). Even though findings are inconsistent, reduced sIgA concentrations after relaxation and meditative practices have been generally found (Heckenberg, Hale, Kent, & Wright, 2019; Tsujita & Morimoto, 1999; Valdimarsdottir & Stone, 1997). The direction of changes in sIgA may be influenced by the population examined and the timing of salivary collections after relaxation and meditation practice (Hewson-Bower & Drummond, 1996; Taniguchi, Hirokawa, Tsuchiya, & Kawakami, 2007). It seems that relaxation may have a modulating effect on sIgA, characterised by increased concentrations in individuals with low sIgA and reduced concentrations in people with high sIgA. Whether sIgA concentrations are elevated or depressed in people with sleep disturbances has not been adequately investigated, although in a study on pregnant women with pregnancy-induced hypertension and gestational diabetes, sIgA concentrations increased from the second and third trimester, and this was inversely correlated with sleep quality (Hayase, Shimada, & Seki, 2014). In another study, sleep deprivation for two nights in healthy males increased concentrations of salivary sIgA (Costa et al., 2010). In relation to sAA, there is an increasing body of evidence confirming it as a valid and reliable marker of stress and autonomic nervous system activity. Alpha-amylase is a salivary enzyme involved in the digestion of carbohydrates and starches but may also reflect central noradrenergic activity (Ali & Nater, 2020). In several studies, mind–body interventions such as stress reduction programs, self-compassion training, and mindfulness-based interventions have been shown to reduce sAA concentrations (Arch et al., 2014; Duchemin et al., 2015; Limm et al., 2011). The changes in morning salivary cortisol concentrations (as evidenced by reductions in morning cortisol in the placebo group) seem to contrast with the findings of the lowering-effects of *Bacopa monnieri* on sIgA and sAA. However, given the significant diurnal activity of cortisol and findings of both hyper- and hypo-cortisol output across different disorders and stress exposures, this finding needs to be interpreted cautiously. Even though lowered cortisol is typically viewed as a positive health-related finding, the research indicates that balance is the key. That is, cortisol concentrations at both the highest and lowest ends predict future health and disease outcomes (Turner et al., 2020). Cortisol concentrations are also significantly influenced by the timing of collection, and in this study, participants were asked to collect saliva samples 30 min after waking. Morning salivary cortisol concentrations are influenced by the cortisol awakening response (CAR) which is typified by an increase of between 38% and 75% in cortisol concentrations 30 to 45 min after awakening (Elder, Wetherell, Barclay, & Ellis, 2014; Fries, Dettenborn, & Kirschbaum, 2009). Delayed or early collections will, therefore, affect findings, and despite participants being instructed to collect samples 30 min after waking, this could not be enforced or reliably monitored. For many participants, waking time was also difficult to determine due to their poor sleep patterns. The CAR also varies significantly across conditions with both higher and lower CAR associated with health benefits. For example, in patients with sleep disturbance and treatment-resistant major depression, a lowered CAR was correlated with more severe depressive symptoms and worse sleep quality (Santiago et al., 2020). In a *meta*-analysis on adults with post-traumatic stress disorder, morning salivary cortisol concentrations were confirmed to be lower (Meewisse, Reitsma, de Vries, Gersons, & Olff, 2007). In another *meta*-analysis, the CAR was positively associated with job stress and general life stress but was negatively associated with fatigue, burnout, and exhaustion (Chida & Steptoe, 2009). Moreover, in a study on adults with chronic fatigue syndrome, lower salivary morning cortisol concentrations were identified (Nater et al., 2008). Adverse early life stressors are also associated with hypocortisolism in adulthood (Juruena, Eror, Cleare, & Young, 2020). These findings suggest that the relationship between morning cortisol and mental and physical wellbeing is not simple; therefore, further research is required to help elucidate the relevance of these findings. Due to cortisol's anti-inflammatory effects, it is important to highlight the finding of reduced pain symptoms in people on *Bacopa monnieri*. Further investigation is required but a potential mechanism associated with bacopa's analgesic effects may be via its effects on cortisol (Hannibal & Bishop, 2014). Interestingly, despite the findings of reduced pain, and differences in changes in cortisol concentrations in the placebo and *Bacopa monnieri* groups, there were no changes in the inflammatory marker, CRP. This may be due to the method of measurement used in this study (saliva versus the more commonly-used blood sample), CRP being an acute-phase inflammatory protein, and/or the potential of recruiting participants with low baseline concentrations of CRP, thereby increasing the possibility of floor effects (Sproston & Ashworth, 2018).

5. Conclusions

In conclusion, in this 28-day, randomised, double-blind, placebo-controlled trial, *Bacopa monnieri* supplementation did not improve sleep patterns more than the placebo in adults with self-reported sleep problems. However, based on findings from secondary outcomes measures, *Bacopa monnieri* was associated with greater improvements in physical and emotional health as measured by the SF-36. In particular, there were improvements in emotional wellbeing, general health and bodily pain subscale scores. An analysis of changes in hormone concentrations over time also revealed *Bacopa monnieri* was associated with reductions in sIgA, sAA, and differences in morning concentrations of salivary cortisol between the two groups (as evidenced by mostly decreased concentrations in the placebo group). Future clinical trials on specifically-recruited populations will be required to validate these secondary findings. To examine the potential effects of *Bacopa monnieri* on sleep, future trials using different doses, dosing regimens, treatment durations, outcome measures (e.g., sleep actigraphy), and adults with varying sleep-related difficulties (e.g., sleep-onset versus sleep-maintenance insomnia) may be helpful. *Bacopa monnieri* is traditionally used as a nootropic herb (Aguiar & Borowski, 2013), and in this study there was some evidence to suggest an increase in morning alertness as demonstrated by an 18% increase in alertness ratings, compared to only 6% in the placebo group (see Supplementary File, Figure S1). This finding will be important to investigate in future trials. Due to the positive findings of *Bacopa monnieri* on mood and stress-related hormones, further investigations into the anxiolytic effects of *Bacopa monnieri* may be warranted using stressed or anxious populations and/or using stress reactivity models such as the Trier Social Stress Test or Maastricht Acute Stress Test (Allen et al., 2017; Smeets et al., 2012).

6. Clinical Trial Registration

This study was prospectively registered with the Australian New Zealand Clinical Trials Registry (Trial ID. ACTRN12620000770965). https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=379967

Funding

This study was funded by Verdure Sciences Inc. Verdure Sciences was not involved in the collection and analysis of data, or the writing of the report.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Dr Lopresti is the managing director of Clinical Research Australia, a contract research organisation that has received research funding from nutraceutical companies. Dr Lopresti has also received presentation honoraria from nutraceutical companies. Mr Smith is an employee of Clinical Research Australia and declares no other conflicts of interest. Dr Kalns is the Vice-President and Chief Scientific Officer of Hyperion Biotechnology, Inc. who developed the testing methods for the Fatigue Biomarker Index. No other authors declare any conflicts of interest]

Acknowledgements

The authors gratefully acknowledge Verdure Sciences Inc for funding the project and supplying capsules used for this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2021.104671.

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