



An Examination into the Anti-stress and Anti-fatigue Effects of an Ashwagandha Extract (Ashwa.30™) in Stressed Adults: A Randomised, Double-Blind, Placebo-controlled Trial

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ABSTRACT

Introduction: Ashwagandha supplementation has been associated with reduced self-reported stress and anxiety, although most trials have used doses of 250–600 mg daily for 6–8 weeks. This study examined the effects of a low-dose ashwagandha extract (Ashwa.30™) on perceived stress, mood, fatigue, and stress reactivity in stressed adults.

Methods: In this 28-day, randomised, double-blind, placebo-controlled trial, 60 adults aged 18–65 years with self-reported stress were randomly allocated to receive ashwagandha 30 mg/

day or placebo. Outcomes included the Depression, Anxiety, and Stress Scale, daily mood ratings, and the Chalder Fatigue Scale. The Socially Evaluated Cold-Pressor Test (SECPT) was conducted on Days 7 and 28, with subjective stress and fatigue, cardiovascular and electrodermal measures, skin temperature, and salivary cortisol and chromogranin A assessed.

Results: Compared to the placebo, ashwagandha intake was associated with greater reductions in self-reported stress in the full analysis set (primary analysis; $p = 0.046$). The per protocol set analysis was directionally consistent but not statistically significant. Mean daily stress ratings were also lower in the ashwagandha group ($p = 0.043$). During the Day 28 SECPT, the ashwagandha group reported lower stress ratings ($p = 0.044$), and salivary cortisol concentrations remained relatively stable in the ashwagandha group but increased in the placebo group, suggesting attenuation of cortisol reactivity ($p = 0.047$). However, no statistically significant between-group differences were detected for other secondary and physiological outcomes.

Conclusions: An ashwagandha extract (Ashwa.30™) administered at 30 mg daily was associated with reduced self-reported stress in the Full Analysis Set and attenuation of SECPT-related salivary cortisol reactivity. These preliminary findings support further investigation in larger, adequately powered trials.

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Keywords: Ashwagandha; *Withania somnifera*; Stress; Fatigue; Mood; HPA-axis; Clinical trial

Key Summary Points

Why carry out this study?

Ashwagandha has demonstrated anti-stress effects in previous clinical trials, but most studies have evaluated doses of 250–600 mg/day administered for 6–8 weeks

Whether a substantially lower dose administered over a shorter treatment period provides clinically meaningful anti-stress effects remains uncertain

Does Ashwa.30™ (30 mg/day) administered for 28 days reduce perceived stress, improve mood and fatigue, and attenuate physiological stress responses compared with placebo in adults experiencing perceived stress?

What was learned from the study?

Compared with placebo, Ashwa.30™ significantly reduced self-reported stress (primary outcome), lowered daily stress ratings, and attenuated salivary cortisol reactivity during an experimental stress challenge after 28 days

No statistically significant between-group differences were observed for most secondary mood, fatigue, or physiological outcomes

These findings suggest that low-dose Ashwa.30™ may reduce perceived stress despite using a substantially lower dose and shorter treatment duration than previous studies, supporting further evaluation in larger confirmatory trials

INTRODUCTION

Ashwagandha is a medicinal plant that has been used in Ayurvedic and indigenous medicine for over 3000 years. In preclinical studies, ashwagandha and its components have been shown to have anti-inflammatory, anti-cancer, anti-stress, immunomodulatory, anti-oxidant, adaptogenic, neuroprotective, and endocrinological activities [1–2]. However, ashwagandha is more typically used for its stress-reducing and anxiolytic effects. In a recently published systematic review and meta-analysis, it was concluded that, based on the results of nine randomised controlled studies involving 558 adults, ashwagandha was more effective than a placebo in reducing self-reported stress and anxiety [3]. Moreover, it was concluded that it lowered serum cortisol concentrations, which was also confirmed in a previously published systematic review [4]. A range of extracts, comprising root-alone and combined root-and-leaf preparations, has been investigated, with doses typically ranging from 250 to 600 mg daily. Administration periods generally range from 6 to 8 weeks.

Ashwa.30™ is an ashwagandha root extract that has been demonstrated as having anxiolytic effects at doses significantly lower than those used in previous trials. In an animal study, reductions in corticosterone and stress-related behaviours were identified in rats exposed to various experimental stressors. A pilot human trial also reported lower post-stressor salivary cortisol concentrations after 7 days of treatment in adults exposed to an acute stress challenge. Therefore, given these positive, preliminary findings, the purpose of this study was to investigate the anti-stress and anti-fatigue effects of Ashwa.30™ in stressed adults at a daily dose of 30 mg for 28 days. Although ashwagandha has been shown to act through multiple mechanisms, it is described as an adaptogen that influences the stress response, particularly the hypothalamic–pituitary–adrenal (HPA) axis [5–7]. Therefore, to investigate its effects on stress reactivity, changes in self-reported and physiological responses upon exposure to a validated experimental procedure, the Socially Evaluated Cold-Pressor Test (SECPT) was used [8]. This included

examining changes in blood pressure, pulse rate, skin conductance, skin temperature, salivary cortisol, and salivary chromogranin A. Changes in heart rate and blood pressure are commonly observed during times of stress [9]. Skin conductance is a measure of the electrical conductivity of the skin, which varies with changes in moisture/sweat levels. Changes in skin conductance/sweat response are often observed during times of stress [10]. Acute stress triggers peripheral vasoconstriction, resulting in a rapid, short-term decrease in skin temperature [11]. Changes in salivary cortisol are regularly observed during stress and are a common physiological measure of stress [12]. Moreover, salivary chromogranin A is a marker of sympathetic–adrenal–medullary activity, as it is co-released with adrenaline or noradrenaline upon sympathetic stimulation [13 14]. It was hypothesised that an ashwagandha extract at 30 mg for 28 days would be associated with reduced self-reported stress, improvements in general mood and fatigue, and reduced subjective and physiological stress responses during the SECPT.

METHODS

Study Design and Procedures

As outlined in Fig. 1, this 28-day trial comprised a two-arm, parallel-group, randomised, double-blind, placebo-controlled design. The study was conducted at Clinical Research Australia in Perth, Western Australia, and received ethical approval from the National Institute of Integrative Medicine Human Research Ethics Committee (approval number: 0153E_2025). Interested volunteers completed a screening questionnaire and, if they were potentially eligible, had a phone interview with an investigator to obtain further information regarding the eligibility criteria. Eligible participants attended an assessment visit (visit 1, Day 0) 3–10 days after the phone interview. During visit 1, the following tasks were completed: (1) completion of the informed consent form, (2) measurement of blood pressure, height, and weight, (3) participant completion of self-report questionnaires,

and (4) 28-day supply of the investigational product (ashwagandha or placebo). Participants attended visit 2 and visits 3, 7 and 28 days later, respectively, when they had their resting vitals measured, completed self-report questionnaires, and underwent the SECPT. Furthermore, participants completed online self-report questionnaires on Day 14 and 21 and, every evening at 20:00, rated their overall stress, anxiety, and depression.

Randomisation and Blinding

Using an in-house database and social media advertising, recruitment was undertaken from March to May 2025. Eligible participants were assigned randomly to either a placebo or an ashwagandha condition in a 1:1 ratio using randomisation software. Block randomisation was used, with six blocks of ten participants each. The randomisation schedule was generated by a research team member who was not involved in participant recruitment, eligibility assessment, outcome assessment, or statistical analyses. Participant identification numbers were assigned sequentially according to enrolment order, and participants received the next available numbered bottle.

Allocation concealment was maintained by using identically appearing active and placebo capsules packaged in matching bottles. Bottle codes were retained by the study sponsor, who was not involved in participant recruitment, outcome assessment, data collection, or statistical analyses. Participants, investigators, outcome assessors, and the statistician remained blinded to treatment allocation until all outcome data had been collected, a blind data review had been completed, and the database had been locked.

Participants

Inclusion criteria

Inclusion criteria for the study comprised the following: adults (female and male) aged between 18 and 65 years; experiencing self-reported perceived stress (mild to severe category) as indicated by a raw score between 8

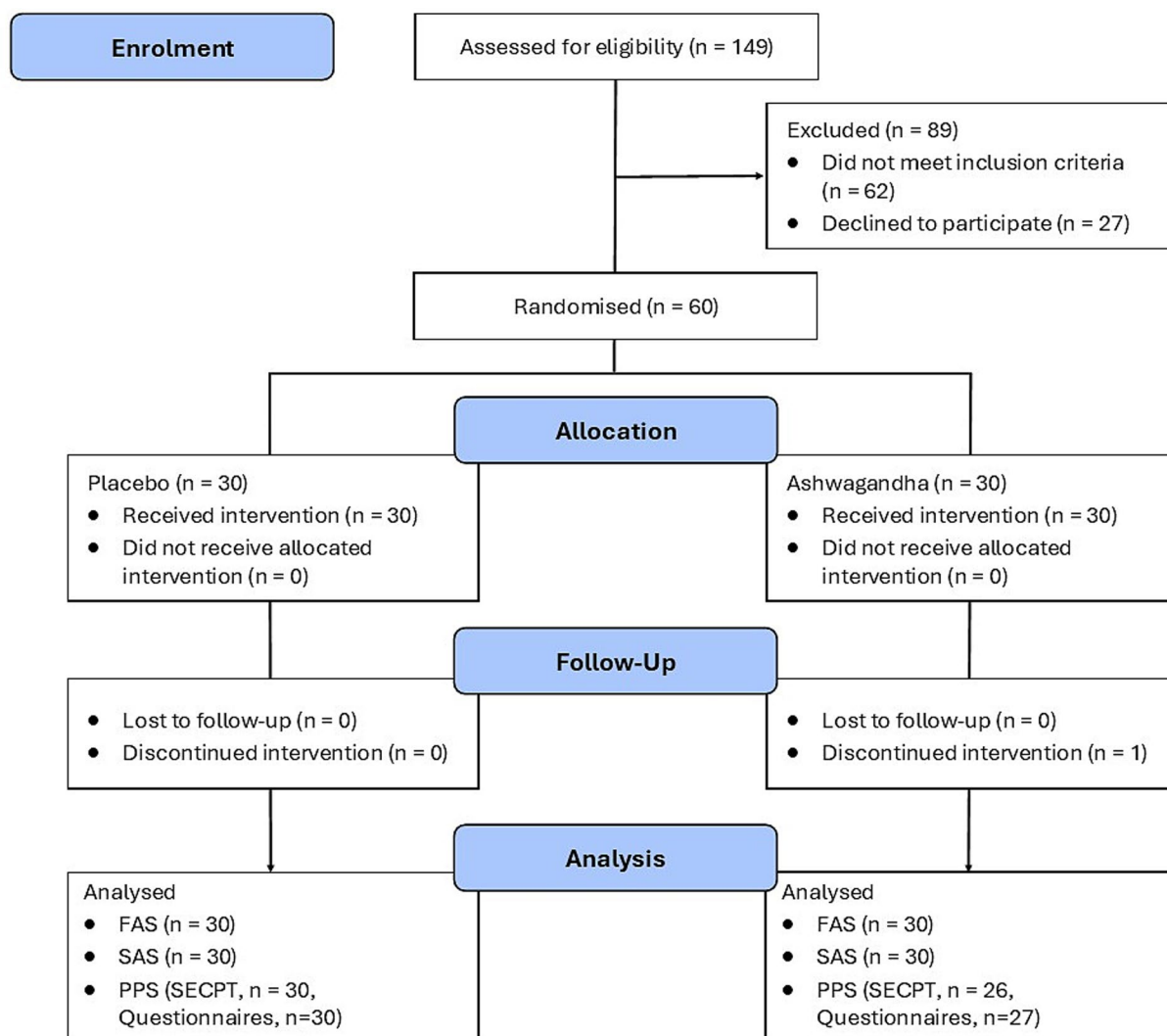


Fig. 1 CONSORT flow diagram of participant progress through the trial. *FAS* full analysis set, *PPS* per protocol set, *SAS* safety analysis set, *SECPT* = socially evaluated cold pressor test

and 16 (converted score 16–32) on the Depression, Anxiety and Stress Scale (DASS-21) stress subscale; were a non-smoker; had a body mass index (BMI) between 18 and 35 kg/m²; and had no intention to start new interventions during the study.

Exclusion criteria

The exclusion criteria consisted of the following: was recently diagnosed or had an unmanaged medical condition, including but not limited to hypertension, diabetes, autoimmune disease,

cardiovascular disease, endocrine disease, pain condition, or malignancy/ cancer; had a mental health diagnosis (other than mild or moderate severity of anxiety or depression) and/or a neurological disease; regularly used medications including but not limited to anti-convulsants, opioids, benzodiazepines, corticosteroids, or immunosuppressants; changed medication in the last 3 months or planned to change during the study; currently used supplements that contained ashwagandha or other ingredients that may influence treatment outcomes; in the last month, changed the dose of, or started a herbal

or nutritional supplement that may influence study outcomes; planned to make a significant change in lifestyle in the next 2 months; consumed more than 14 standard alcoholic drinks per week; currently (or in the last year) recurrently used illicit drugs; was pregnant, breastfeeding, or planned to become pregnant during the study; or had any major surgeries over the last 12 months.

Plant Material and Interventions

The intervention consisted of capsules containing an ashwagandha root extract (NRAHG001), known as Ashwa.30™, or a matching placebo (microcrystalline cellulose). The roots of *Withania somnifera*, popularly known as Ashwagandha, a shrub belonging to the Solanaceae family, were sourced from Andhra Pradesh, India. The authentication was performed, and voucher specimen number 128101 is kept in the Herbarium of the Foundation for Revitalisation of Local Health Traditions, Bengaluru. The test substance used is a standardised proprietary extract (pending patent: 202541047928) derived from the roots of *Withania somnifera*. It is standardised to withanolides (>15% w/w) and an ATP-active fraction (>15% w/w) [15]. Participants took one capsule in the morning with or without food, with the active intervention delivering 30 mg of Ashwa.30™ daily for 28 days. The placebo and active capsules were similar in size, shape, and colour, and both contained the same excipients (microcrystalline cellulose IP101 and syloid 244FP). Participants returned their bottle at visits 2 and 3, and a capsule count was undertaken at each visit to evaluate treatment compliance. To assess the efficacy of treatment blinding, participants predicted their group allocation (placebo, ashwagandha, or unsure) and the reasons for their predictions at visit 3.

Outcome Measures

Depression, Anxiety, and Stress Scale–21 (DASS-21) The DASS-21 is a psychometrically-validated self-report inventory that assesses affective symptoms over the last 7 days [16]. It consists of 21 items, where subscale scores for depression,

anxiety, and stress were computed. Raw scores are doubled, and a converted score of 16–32 on the DASS-21 stress subscale indicates a mild-to-severe stress severity. The DASS-21 stress score was designated as the primary outcome measure, and the depression and anxiety scores as secondary outcome measures.

Socially evaluated cold-pressor test (SECPT) The SECPT is a validated experimental procedure designed to elicit a stress response in individuals [8]. Participants were required to place their non-dominant hand in cold water for up to 3 min. The water temperature was maintained at 7 °C using a refrigerated water bath. To increase the stress response during the procedure, participants were also led to believe that their facial expressions were being recorded. The SECPT was conducted on Days 7 and 28, and participants were instructed to refrain from consuming food or drink for 1 h prior to each visit, not to consume alcohol on the day of testing, and to refrain from consuming any caffeinated beverages for 4 h before the SECPT. The following measures were collected:

1. Salivary collections: Salivary cortisol and chromogranin A were measured immediately before the SECPT and 30 min after the SECPT. Unstimulated passive drool was collected into polypropylene microtubes and immediately stored in a – 20 °C freezer for later analysis. To control for diurnal cortisol fluctuations, all assessments were conducted between 13:00 and 17:00. Participants also refrained from drinking water for at least 5 min prior to each saliva collection.
2. Blood pressure: Using an automatic blood pressure monitor, blood pressure was measured immediately before the SECPT, immediately after the SECPT, and 10, 20 and 30 min after the SECPT.
3. Visual analogue scale (VAS) ratings: Subjective feelings of stress and mental fatigue were assessed immediately before the SECPT, immediately after the SECPT, and 10, 20 and 30 min after the SECPT using a VAS consisting of a 100-mm horizontal line with the ends of the line labelled “not at all” (0 mm, on the left) and “extremely” (100 mm, on the right). Participants were asked to mark

on the line ‘how stressed they feel right now’ and ‘how mentally fatigued they feel right now’.

4. Continuous physiological biomarkers: Participants wore the EmbracePlus wristband during and for 30 min after the SECPT. The EmbracePlus is part of the FDA-cleared Empatica Health Monitoring Platform that has been used in several studies to measure physiological biomarkers [17 18]. Pulse rate was measured using optical photoplethysmography, skin temperature using a peripheral temperature sensor, and skin conductance using ventral electrodermal activity sensors.

Chalder Fatigue Scale (CFS) The CFS is a validated 11-item self-report questionnaire that measures fatigue and low-energy symptoms [19 20]. Each question is rated using a 4-point scale. The original CFS refers to the last month; however, in this study, participants were asked to provide ratings based on the last 7 days.

Single Item Mood Rating (SIMR) To assess mood variability over 28 days, participants completed a daily SIMR for (1) stress, (2) anxiety, and (3) depression. Each question was taken from each DASS-21 subscale, comprising the following questions: ‘Today I found it difficult to relax’ (stress), ‘Today I felt downhearted and blue’ (depression), and ‘Today I felt scared without any good reason’ (anxiety). These questions are highly correlated with their respective subscale total scores [21].

Safety and Expectancy Measures

Tolerance to capsule intake was evaluated through weekly questionnaires enquiring about adverse events and an interview at visits 2 and 3. Participants indicated their tolerance to the capsules by completing the Patient Global Assessment of Tolerability to Therapy (PGATT) at visit 3. Ratings ranged from ‘Worst—I experienced severe discomfort and was unable to tolerate the treatment’ to ‘Excellent—I experienced no discomfort or adverse effects.’ Seated blood pressure, resting pulse, and weight were also measured at each visit. At visit 1, participants also completed the Clinical Trials Treatment

Expectancies Scale (CTTES). The CTTES is a 6-item questionnaire used to measure and control for treatment-based expectancies [22].

The DASS-21, SECPT and CFS scales are established academic research instruments that do not require formal permission for use in academic research. The SIMR and CTTES were developed by the authors. Accordingly, no third-party permissions were required.

Sample Size Calculations

Previous meta-analyses have reported moderate-to-large improvements in stress outcomes following ashwagandha supplementation. However, reported effect sizes vary substantially across studies due to differences in extracts, doses, populations, treatment durations, outcome measures and placebo responses [3]. For the present study, an effect size of 0.7 was used as a pragmatic, evidence-informed planning assumption to detect a moderate-to-large treatment effect on the primary outcome measure. This estimate was informed by previous ashwagandha trials and meta-analytic findings, while recognising that previous Australian ashwagandha studies, including studies conducted by our group, have reported substantial placebo responses and more modest between-group effects [23 24]. Based on a power of 80%, a 5% two-sided alpha error rate, and an assumed effect size of 0.7, a total sample of 52 participants was required. Allowing for an anticipated dropout rate of approximately 10%, the planned sample size was 60 participants.

Statistical Analysis

Outcome analyses were conducted using the full analysis set (FAS), defined as all randomised participants who took at least one dose of the investigational product and had available efficacy data. Sensitivity analyses were conducted using the per protocol set (PPS), comprising FAS participants with no major protocol deviations. Safety analyses included all participants who took at least one dose of the investigational product.

Self-report outcomes, including DASS-stress, DASS-anxiety, DASS-depression, and Chalder Fatigue Scale scores, were analysed using repeated-measures mixed-effects models. Separate models were conducted for each outcome, with post-baseline scores at Days 7, 14, 21 and 28 included as repeated outcome measures. Treatment group, time and the treatment group-by-time interaction were included as fixed effects. The corresponding baseline score, age, sex, BMI and CTES positive and negative expectancy scores were included as covariates. Participant ID was included as the repeated-measures subject variable, and a compound symmetry covariance structure was used. Estimated marginal means and standard errors were calculated for each treatment group at each post-baseline time point. The Day 28 between-group comparison represented the primary post-treatment comparison for the primary outcome.

Daily SIMR for stress, anxiety, and depression were averaged within each treatment week and analysed using repeated-measures mixed-effects models. Separate models were conducted for each SIMR outcome, with treatment group, week, and the treatment group-by-week interaction included as fixed effects. Age, sex, BMI, CTES positive and negative expectancy scores, and the corresponding baseline DASS-21 subscale score were included as covariates.

SECPT outcomes were analysed using repeated-measures mixed-effects models separately for each outcome and assessment day. Subjective, cardiovascular, and EmbracePlus-derived measures were analysed using values obtained before, immediately after, and 10, 20, and 30 min after the SECPT. Salivary cortisol and chromogranin A were analysed using values obtained before the SECPT and 30 min after. Models included treatment group, SECPT time point, and the treatment group-by-SECPT time point interaction as fixed effects, with participant ID included as the repeated-measures subject variable. Estimated marginal means and standard errors were calculated at each SECPT time point.

Within-group changes were examined using pairwise comparisons of estimated marginal means. Cohen's *d* values were calculated for Day 28 between-group differences using adjusted

mean differences divided by the model-based standard deviation. All statistical tests were two-sided, with $p < 0.05$ considered statistically significant. As only one a priori primary outcome was specified, no adjustment was made for multiple comparisons. Secondary outcomes, SIMR outcomes, SECPT outcomes, and within-group analyses were therefore interpreted as exploratory. Analyses were conducted using SPSS version 30.0 (IBM, Armonk, NY, USA).

RESULTS

Study population

As detailed in Fig. 1, 149 people completed the online screening questionnaire and 60 were randomised. Overall, 89 were excluded, most commonly because they did not meet the inclusion criteria ($n = 62$) or declined to participate ($n = 27$). Baseline clinical and sociodemographic characteristics, as well as mean scores at Visit 1, are presented in Table 1.

Outcome measures

Stress scores As shown in Table 2 and Fig. 2, the repeated-measures mixed-effects model indicated a statistically significant adjusted between-group difference in DASS-stress scores on Day 28, favouring ashwagandha. The adjusted mean DASS-stress score at Day 28 was 10.47 in the ashwagandha group and 13.06 in the placebo group, corresponding to an adjusted mean difference of 2.60 points in favour of ashwagandha (95% CI 0.04, 5.15; Cohen's $d = 0.55$; $p = 0.046$). Within-group analyses from Day 0 to Day 28 indicated significant reductions in DASS-stress scores in both the ashwagandha and placebo groups ($p < 0.001$ for both groups). After controlling for baseline scores (mean: 19.45), the DASS-21 stress score decreased by 46.2% in the ashwagandha group, and by 32.9% in the placebo group. In the PPS analysis, the adjusted Day 28 between-group difference in DASS-stress scores was directionally consistent with the FAS analysis but did not reach statistical significance. At Day 28, the adjusted mean DASS-stress score was

Table 1 Baseline sociodemographic and clinical characteristics

		Placebo (<i>n</i> = 30)	Ashwagandha (<i>n</i> = 30)
Age (years)	Mean	47.17	40.43
	SD	12.09	13.90
Sex (<i>n</i>)	Male	7	10
	Female	23	20
Height (m)	Mean	1.67	1.68
	SD	0.08	0.10
Weight (kg)	Mean	71.25	74.16
	SD	13.40	16.28
BMI (kg/m ²)	Mean	25.57	26.12
	SD	3.92	4.47
Exercise days per week	Mean	2.50	1.97
	SD	1.97	2.20
Systolic blood pressure (mmHg)	Mean	119.00	120.27
	SD	10.72	16.15
Diastolic blood pressure (mmHg)	Mean	76.70	76.90
	SD	6.80	10.90
Pulse (bpm)	Mean	70.63	73.23
	SD	9.73	10.80
Marital status (<i>n</i>)	Single	15	17
	Married/defacto	15	13
Education (<i>n</i>)	Secondary	10	17
	Tertiary	16	10
	Post-graduate	4	3

Table 1 continued

		Placebo (<i>n</i> = 30)	Ashwagandha (<i>n</i> = 30)
Occupation (<i>n</i>)	Technicians and associated trades	8	6
	Student	3	6
	Clerical support worker	5	4
	Unemployed	2	4
	Professional	4	1
	Manager	2	3
	Elementary occupation	2	3
	Services and sales worker	2	2
	Craft and related trades worker	2	0
	Retired	0	1
DASS-stress	Mean	18.73	20.07
	SD	7.08	6.84
DASS-depression	Mean	10.53	12.20
	SD	7.28	7.13
DASS-anxiety	Mean	9.07	9.33
	SD	6.84	5.49
CFS	Mean	16.43	16.57
	SD	4.23	4.99
CTTES positive expectancies	Mean	10.03	9.40
	SD	2.16	2.33
CTTES negative expectancies	Mean	3.93	1.53
	SD	1.53	1.31

10.99 in the ashwagandha group and 13.02 in the placebo group, corresponding to an adjusted mean difference of 2.04 points in favour of ashwagandha (95% CI -0.51, 4.59; Cohen's $d = 0.44$; $p = 0.116$).

As shown in Table 3, daily SIMR-Stress ratings were averaged within each week and analysed as weekly mean scores. The repeated-measures mixed-effects model indicated no statistically significant group-by-week interaction for SIMR-Stress scores ($p = 0.969$), suggesting that the pattern of change across Weeks 1–4 did not differ significantly between groups. However, there was a statistically

significant overall group effect ($p = 0.043$), indicating lower adjusted weekly mean stress ratings in the ashwagandha group than in the placebo group over the 4-week treatment period. Averaged across Weeks 1–4, adjusted SIMR-Stress scores were 0.69 in the ashwagandha group and 0.87 in the placebo group, corresponding to an overall adjusted mean difference of 0.18 points. Within-group analyses indicated significant reductions in weekly mean SIMR-Stress scores from Week 1 to Week 4 in both the placebo group ($p = 0.006$) and the ashwagandha group ($p = 0.012$).

Table 2 Adjusted self-report outcome scores by treatment group—full analysis set

Outcome	Group	D7 mean (SE)	D14 mean (SE)	D21 mean (SE)	D28 mean (SE)	Within-group Day 0–28 <i>p</i> value	Group × Time <i>p</i> value	D28 difference, placebo-ashwagandha (SE)	Cohen's <i>d</i>	D28 <i>p</i> value
DASS-stress	Placebo	13.73 (0.92)	12.79 (0.93)	12.39 (0.92)	13.06 (0.92)	< 0.001	0.183	2.60 (1.29)	0.55	0.046
	Ashwagandha	13.69 (0.93)	12.13 (0.94)	10.90 (0.95)	10.47 (0.93)	< 0.001				
DASS-anxiety	Placebo	6.05 (0.74)	5.03 (0.74)	4.25 (0.74)	4.72 (0.74)	< 0.001	0.752	0.79 (1.02)	0.21	0.442
	Ashwagandha	6.06 (0.75)	4.25 (0.75)	4.31 (0.76)	3.93 (0.74)	< 0.001				
DASS-depression	Placebo	9.07 (0.90)	7.76 (0.91)	7.87 (0.90)	7.34 (0.90)	< 0.001	0.922	2.21 (1.26)	0.48	0.081
	Ashwagandha	7.34 (0.91)	6.32 (0.92)	6.23 (0.93)	5.13 (0.90)	< 0.001				
CFS	Placebo	12.76 (0.82)	11.62 (0.83)	11.53 (0.82)	10.79 (0.82)	< 0.001	0.158	– 1.85 (1.14)	– 0.44	0.110
	Ashwagandha	12.37 (0.83)	12.48 (0.84)	12.32 (0.85)	12.64 (0.83)	< 0.001				

Values are estimated marginal means and standard errors from repeated-measures mixed-effects models. Day 28 differences are presented as placebo minus ashwagandha. Positive differences favour ashwagandha for DASS outcomes, whereas the negative CFS difference indicates lower adjusted fatigue scores in the placebo group. Within-group Day 0–28 *p* values were obtained from separate within-group mixed-effects models. Cohen's *d* values represent standardised Day 28 between-group differences. *P* values were not adjusted for multiple comparisons.

CFS Chalder Fatigue Scale, DASS Depression Anxiety Stress Scales, SE standard error

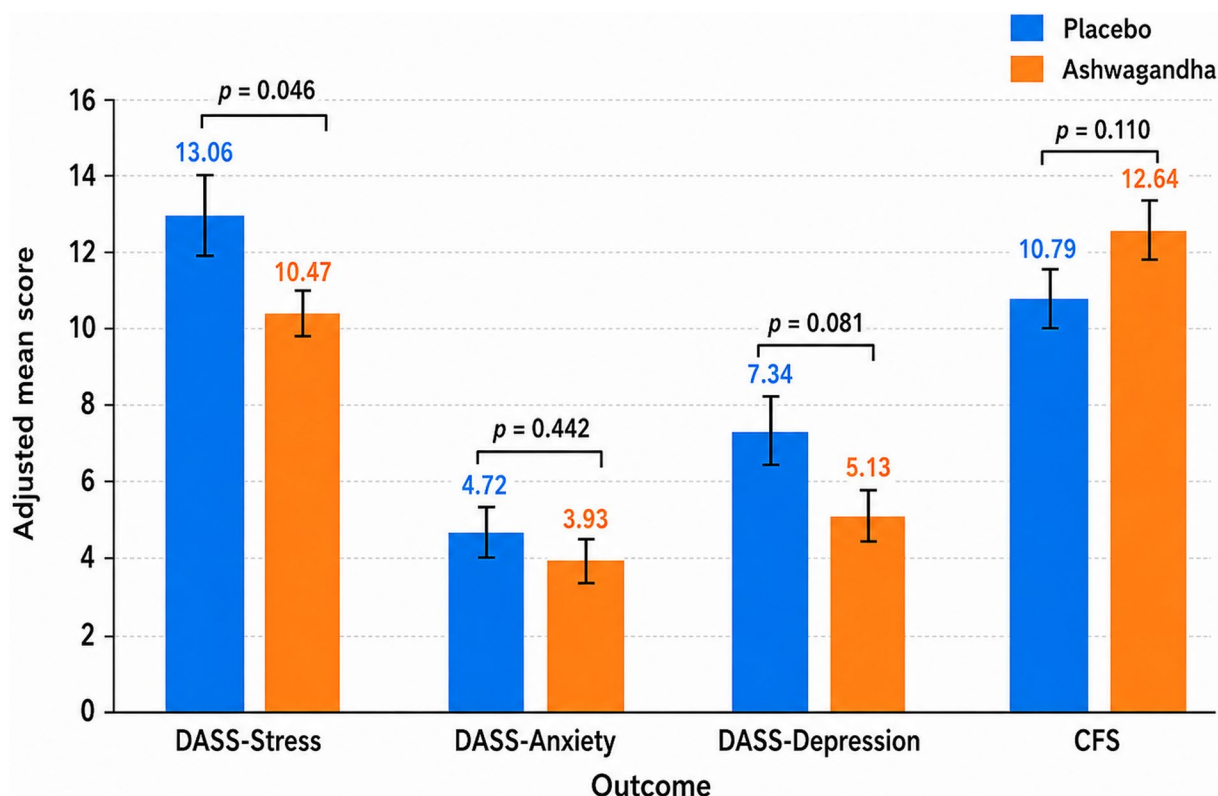


Fig. 2 Adjusted mean Day 28 outcome scores by treatment group (full analysis set). Adjusted means were estimated after controlling for baseline score, age, sex, body mass index, and Clinical Trials Treatment Expectan-

cies Scale positive and negative expectancy scores. *Error bars* represent standard errors; *p* values indicate adjusted between-group comparisons at Day 28. Lower scores indicate fewer symptoms or lower fatigue

Anxiety scores As demonstrated in Table 2 and Fig. 2, the repeated-measures mixed-effects model indicated no statistically significant adjusted between-group difference in DASS-anxiety scores on Day 28. The adjusted mean DASS-anxiety score at Day 28 was 3.93 in the ashwagandha group and 4.72 in the placebo group, corresponding to an adjusted mean difference of 0.79 points in favour of ashwagandha (95% CI -1.24, 2.82; Cohen's $d = 0.21$; $p = 0.442$). Within-group analyses from Day 0 to Day 28 indicated significant reductions in DASS-anxiety scores in both the ashwagandha and placebo groups ($p < 0.001$ for both groups). After controlling for baseline scores (mean: 9.34), the DASS-anxiety score decreased by 57.9% in the ashwagandha group and by 49.5% in the placebo group. Similar non-significant

between-group findings were identified based on the PPS analysis (Supplementary Table S1).

As shown in Table 3, daily SIMR-anxiety ratings were averaged within each week and analysed as weekly mean scores. The repeated-measures mixed-effects model indicated no statistically significant overall group effect for SIMR-anxiety scores ($p = 0.780$), suggesting no statistically significant adjusted difference between the ashwagandha and placebo groups over the 4-week treatment period. The group-by-week interaction was also not statistically significant ($p = 0.064$), although the pattern of weekly change differed numerically between groups. Within-group analyses indicated a significant reduction in weekly mean SIMR-anxiety scores from Week 1 to Week 4 in the ashwagandha group ($p = 0.023$), whereas no statistically

Table 3 Adjusted weekly SIMR scores by treatment group—full analysis set

		Placebo ($n = 30$)				Ashwagandha ($n = 30$)				Group $\times$ week		Overall group
		Week 1	Week 2	Week 3	Week 4	Within-group Week 1–4 p value	Week 1	Week 2	Week 3	Week 4	Within-group Week 1–4 p value	p value
SIMR-stress	Mean	1.03	0.92	0.77	0.75	0.006	0.83	0.79	0.58	0.57	0.012	0.043
	SE	0.09	0.09	0.09	0.09		0.08	0.09	0.09	0.09		
SIMR-anxiety	Mean	0.33	0.22	0.32	0.28	0.499	0.39	0.38	0.24	0.23	0.023	0.780
	SE	0.08	0.08	0.08	0.08		0.08	0.08	0.08	0.08		
SIMR-depression	Mean	0.55	0.52	0.54	0.53	0.868	0.58	0.49	0.36	0.32	0.004	0.299
	SE	0.09	0.09	0.09	0.09		0.09	0.09	0.09	0.09		

Values are estimated marginal means and standard errors from repeated-measures mixed-effects models. Within-group p values represent Week 1 to Week 4 changes within each treatment group. Group-by-week p values indicate treatment group $\times$ week interactions. Group-effect p values indicate overall adjusted differences between treatment groups across Weeks 1–4; p values were not adjusted for multiple comparisons.

CTTES Clinical Trials Treatment Expectancies Scale, DASS Depression Anxiety Stress Scales, SE standard error, SIMR single-item mood rating

significant change was observed in the placebo group ($p = 0.499$). At Week 4, adjusted SIMR-anxiety scores were 0.23 in the ashwagandha group and 0.28 in the placebo group.

Depression scores As demonstrated in Table 2 and Fig. 2, the repeated-measures mixed-effects model indicated no statistically significant adjusted between-group difference in DASS-depression scores on Day 28. The adjusted mean DASS-depression score at Day 28 was 5.13 in the ashwagandha group and 7.34 in the placebo group, corresponding to an adjusted mean difference of 2.21 points in favour of ashwagandha (95% CI $-0.28, 4.70$; Cohen's $d = 0.48$; $p = 0.081$). Within-group analyses from Day 0 to Day 28 indicated significant reductions in DASS-depression scores in both the ashwagandha and placebo groups ($p < 0.001$ for both groups). After controlling for baseline scores (mean: 11.22), the DASS-depression score decreased by 54.3% in the ashwagandha group and by 34.6% in the placebo group. Similar non-significant between-group findings were identified based on the PPS analysis (Supplementary Table S1).

As shown in Table 3, daily SIMR-depression ratings were averaged within each week and analysed as weekly mean scores. The repeated-measures mixed-effects model indicated no statistically significant overall group effect for SIMR-depression scores ($p = 0.299$), suggesting no statistically significant adjusted difference between the ashwagandha and placebo groups over the 4-week treatment period. The group-by-week interaction was also not statistically significant ($p = 0.162$), indicating that the pattern of weekly change did not differ significantly between groups. Within-group analyses indicated a significant reduction in weekly mean SIMR-depression scores from Week 1 to Week 4 in the ashwagandha group ($p = 0.004$), whereas no statistically significant change was observed in the placebo group ($p = 0.868$). At Week 4, adjusted SIMR-depression scores were 0.32 in the ashwagandha group and 0.53 in the placebo group.

SECPT measures Measures collected during the SECPT on Days 7 and 28 are detailed in Supplementary Table S3 (Day 7 data) and Table 4 (Day 28 data). As detailed in Fig. 3, on Day 28, overall VAS stress ratings were lower in the

ashwagandha group than in the placebo group ($p = 0.044$). Statistically significant group differences in stress ratings were identified immediately after the SECPT and 10 min after the SECPT, where stress ratings were 13.28 ($p = 0.013$) and 9.12 ($p = 0.028$) points lower, respectively, in the ashwagandha group. On Days 7 and 28, statistically significant time-by-group interactions were observed for VAS fatigue ratings during the SECPT assessment period (Day 7: $p = 0.018$; Day 28: $p = 0.005$). These interactions were primarily characterised by higher pre-SECPT fatigue ratings in the ashwagandha group, followed by reductions over the post-SECPT assessment period. In contrast, fatigue ratings in the placebo group remained relatively stable. As participants in the ashwagandha group reported higher fatigue ratings before the SECPT at both visits, these baseline differences limit interpretation of the fatigue trajectories. The fatigue findings should therefore be considered exploratory and should not be interpreted as definitive evidence of a treatment-specific effect on fatigue reactivity. No group differences in pulse rate or blood pressure were identified on Days 7 or 28 (Table 4 and Supplementary Fig. S3). On Day 7, a statistically significant group difference in skin temperature was observed ($p = 0.030$), with the ashwagandha group showing consistently higher skin temperature after the SECPT. However, there were no group differences in skin temperature on Day 28.

In relation to salivary measures (Table 5; Fig. 4), there were no group differences in chromogranin concentrations on Days 7 or 28, or cortisol concentrations on Day 7. However, on Day 28, a statistically significant time-by-group interaction was observed for salivary cortisol concentrations ($p = 0.047$). In the placebo group, cortisol concentrations increased over time. However, in the ashwagandha group, cortisol concentrations remained relatively constant. Thirty minutes after the SECPT, cortisol concentrations were significantly higher in the placebo group compared to the ashwagandha group ($p = 0.040$).

CFS scores As shown in Table 2 and Fig. 2, there was no statistically significant adjusted between-group difference in CFS scores at Day 28 (adjusted mean difference = -1.85 ; 95% CI

Table 4 Day 28 SECPT measures by treatment group—estimated marginal means, full analysis set

			SECPT—Day 28					<i>p</i> value (group x time)	<i>p</i> value (group)
			Before	After	10 min	20 min	30 min		
VAS-stress	Placebo	Mean	21.60	31.33	22.99	19.20	18.07	0.056	0.044
		SE	3.33	4.59	3.51	3.03	2.90		
	Ashwagandha	Mean	16.57	18.05	13.86	13.17	12.10		
		SE	2.69	2.89	2.32	2.23	2.09		
	Group differences	Difference	5.03	13.28	9.12	6.03	5.96	NA	
		SE	4.21	5.34	4.14	3.71	3.52		
		<i>p</i> value	0.233	0.013	0.028	0.105	0.091		
VAS-fatigue	Placebo	Mean	30.84	34.02	32.53	29.20	28.57	0.005	0.957
		SE	4.12	4.36	4.24	4.00	3.95		
	Ashwagandha	Mean	36.62	30.56	30.92	28.14	27.91		
		SE	4.70	4.19	4.22	4.00	3.99		
	Group differences	Difference	− 5.79	3.45	1.62	1.06	0.66	NA	
		SE	6.03	5.85	5.79	5.49	5.44		
		<i>p</i> value	0.338	0.555	0.780	0.846	0.903		
Pulse	Placebo	Mean	69.40	67.75	65.92	65.11	64.45	0.495	0.227
		SE	1.69	1.66	1.62	1.61	1.59		
	Ashwagandha	Mean	72.09	68.70	69.02	68.02	67.56		
		SE	1.73	1.66	1.67	1.65	1.64		
	Group differences	Difference	− 2.70	− 0.95	− 3.10	− 2.91	− 3.12	NA	
		SE	2.38	2.31	2.29	2.27	2.25		
		<i>p</i> value	0.258	0.680	0.178	0.201	0.167		
Systolic BP	Placebo	Mean	119.93	121.74	115.25	115.15	118.51	0.633	0.866
		SE	2.44	2.47	2.38	2.38	2.42		
	Ashwagandha	Mean	117.78	122.79	116.66	116.72	119.09		
		SE	2.41	2.48	2.39	2.39	2.43		
	Group differences	Difference	2.15	− 1.05	− 1.41	− 1.57	− 0.58	NA	
		SE	3.39	3.46	3.34	3.34	3.39		
		<i>p</i> value	0.527	0.762	0.673	0.638	0.864		

Table 4 continued

			SECPT—Day 28						
			Before	After	10 min	20 min	30 min	<i>p</i> value (group x time)	<i>p</i> value (group)
Diastolic BP	Placebo	Mean	80.17	80.28	77.42	78.81	78.82	0.873	0.301
		SE	1.48	1.48	1.45	1.47	1.47		
	Ashwagandha	Mean	78.69	78.84	74.81	76.58	78.04		
		SE	1.46	1.46	1.43	1.44	1.46		
	Group differences	Difference	1.48	1.43	2.61	2.23	0.78	NA	
		SE	2.06	2.06	2.01	2.03	2.04		
		<i>p</i> value	0.472	0.487	0.196	0.274	0.703		
Embrace-pulse	Placebo	Mean	73.16	77.16	70.29	67.96	68.16	0.754	0.999
		SE	2.12	2.24	1.84	1.65	1.70		
	Ashwagandha	Mean	72.79	78.71	71.05	70.69	70.63		
		SE	2.06	2.24	1.82	1.67	1.71		
	Group differences	Difference	0.37	− 1.56	− 0.76	− 2.73	− 2.47	NA	
		SE	2.94	3.14	2.56	2.33	2.39		
		<i>p</i> value	0.899	0.621	0.766	0.242	0.303		
Embrace-EDA	Placebo	Mean	0.23	0.31	0.45	0.18	0.18	0.754	0.999
		SE	0.10	0.10	0.13	0.07	0.07		
	Ashwagandha	Mean	0.19	0.29	0.38	0.25	0.23		
		SE	0.10	0.10	0.13	0.07	0.07		
	Group differences	Difference	0.03	0.01	0.07	− 0.07	− 0.05	NA	
		SE	0.15	0.14	0.19	0.10	0.09		
		<i>p</i> value	0.813	0.925	0.694	0.487	0.557		
Embrace-temperature	Placebo	Mean	29.85	30.51	30.74	30.88	30.90	0.957	0.609
		SE	0.42	0.38	0.35	0.36	0.39		
	Ashwagandha	Mean	30.02	30.75	31.04	31.19	31.23		
		SE	0.41	0.38	0.35	0.36	0.39		
	Group differences	Difference	− 0.16	− 0.24	− 0.29	− 0.31	− 0.33	NA	
		SE	0.59	0.53	0.49	0.51	0.55		
		<i>p</i> value	0.780	0.649	0.551	0.541	0.541		

Values are estimated marginal means and standard errors from repeated-measures mixed-effects models.

SE standard error, SECPT Socially Evaluated Cold Pressor Test

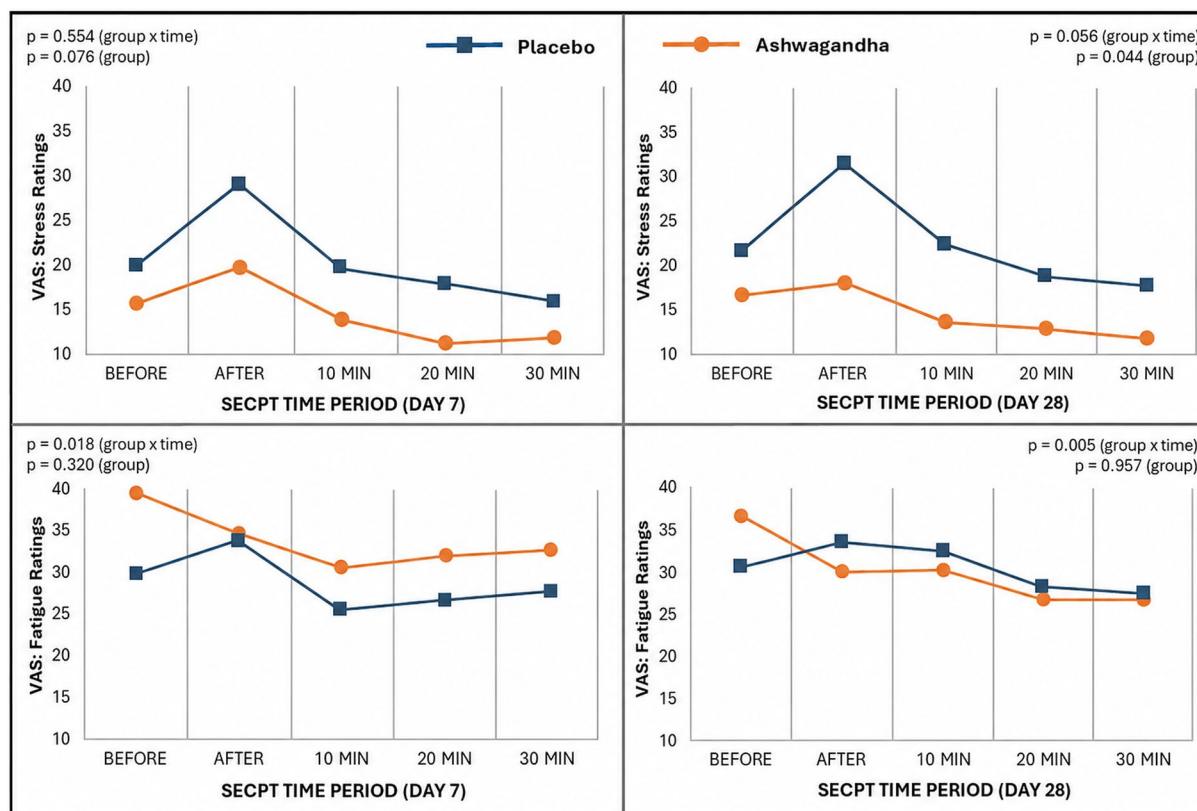


Fig. 3 Change in VAS ratings during the SECPT by treatment group. Values represent estimated marginal means for subjective stress and fatigue ratings before, immediately after, and 10, 20 and 30 min after the SECPT on Days 7 and 28. VAS scores range from 0 to 100, with higher scores indicating greater perceived stress or fatigue; p values are from repeated-measures mixed-effects models and repre-

sent the overall treatment group effect and treatment group $\times$ SECPT time-point interaction. SECPT outcomes were secondary/exploratory and were not adjusted for multiple comparisons. Fatigue findings should be interpreted cautiously because pre-SECPT fatigue ratings differed between groups

– 4.12, 0.43; $p = 0.110$), although scores were numerically lower in the placebo group. Both groups showed significant within-group reductions from Day 0 to Day 28 ($p < 0.001$ for both groups). Similar non-significant findings were observed in the PPS analysis (Supplementary Table S1).

Intake of Supplements

To assess treatment compliance, the researcher conducted a capsule count at the end of the study. Based on this information, 58 of the 59 participants who completed the study took over 90% of their capsules.

Efficacy of Participant Blinding

To evaluate blinding efficacy, participants predicted their treatment allocation (i.e., placebo, ashwagandha, or unsure) at study completion. Group concealment was maintained, as 21 (72.4%) participants in the ashwagandha group and 18 (60.0%) participants in the placebo group were uncertain or incorrectly predicted their treatment allocation. No participants correctly predicted treatment allocation, based on the taste, smell or visual appearance of the capsules.

Table 5 Salivary cortisol and chromogranin A concentrations during the SECPT procedure on Days 7 and 28—estimated marginal means, full analysis set

		SECPT—Day 7				SECPT—Day 28			
		Before	30 min	<i>p</i> value (group x time)	<i>p</i> value (group)	Before	30 min	<i>p</i> value (group x time)	<i>p</i> value (group)
Salivary cortisol (ug/dL)	Placebo	Mean	0.159	0.383	0.876	0.188	0.350	0.047	0.063
		SE	0.022	0.085		0.022	0.051		
	Ashwagandha	Mean	0.148	0.356		0.169	0.201		
		SE	0.022	0.088		0.022	0.052		
	Group differences	Difference	0.011	0.027	NA	0.019	0.150	NA	
		SE	0.030	0.122		0.031	0.072		
Salivary chromogranin A (pg/mL)	Placebo	<i>P</i> -value	0.705	0.824		0.545	0.040		
		Mean	230.27	133.38	0.216	156.28	117.16	0.889	0.936
		SE	43.16	25.00		30.85	23.13		
	Ashwagandha	Mean	165.52	115.24		154.80	113.45		
		SE	30.65	21.34		30.05	22.03		
	Group differences	Difference	64.75	18.14	NA	1.48	3.71	NA	
		SE	52.02	32.27		42.47	31.49		
		<i>P</i> -value	0.216	0.575		0.972	0.906		

Values are estimated marginal means and standard errors from repeated-measures mixed-effects models. Group differences are calculated as placebo minus ashwagandha. Group-by-time *p* values indicate treatment group × SECPT time-point interactions, and group *p* values indicate overall adjusted differences between treatment groups. *P* values shown in the group-difference rows indicate between-group differences at each SECPT time point; *p* values were not adjusted for multiple comparisons.

SE standard error, *SECPT* Socially Evaluated Cold Pressor Test

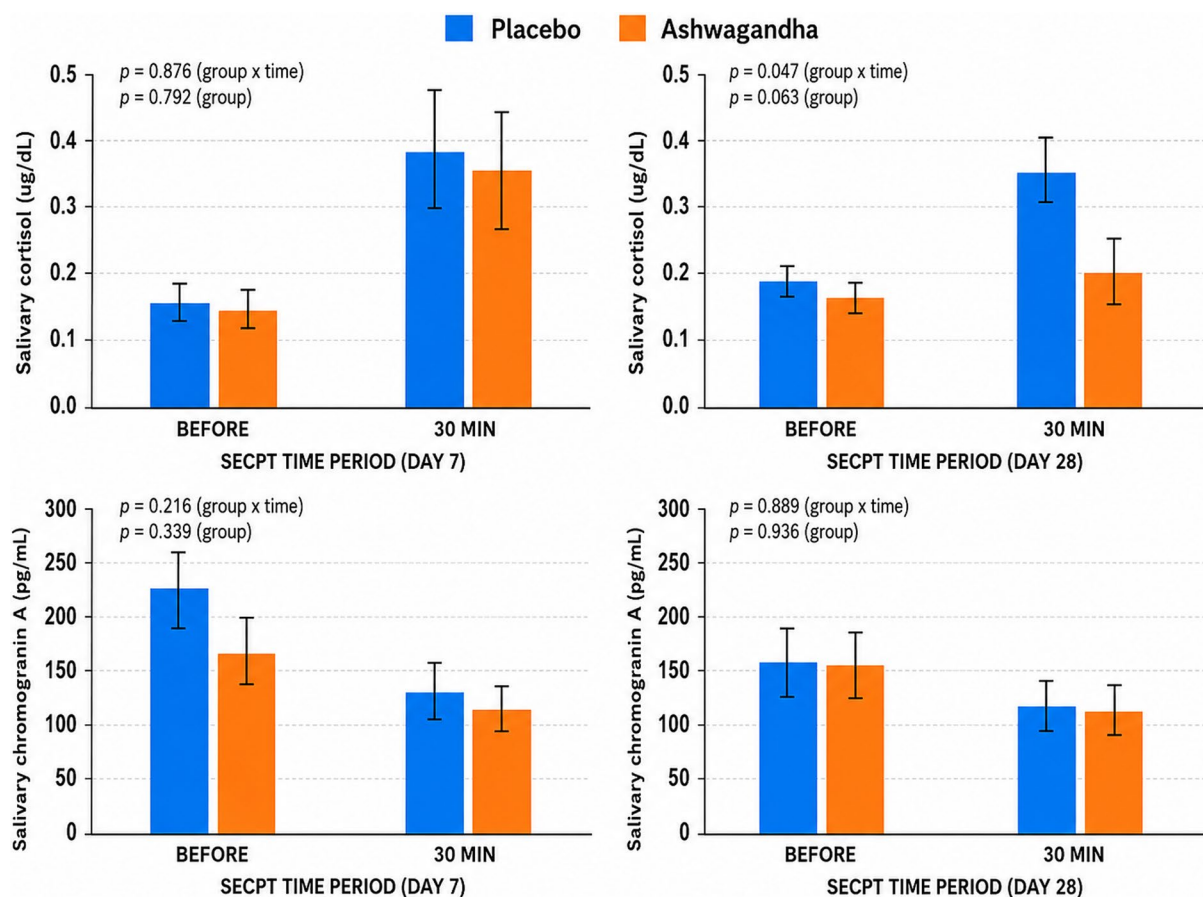


Fig. 4 Change in salivary cortisol and chromogranin A concentrations during the SECPT by treatment group. Values represent estimated marginal means for salivary cortisol and chromogranin A concentrations measured immediately before and 30 min after the SECPT on Days 7 and 28. Error bars represent standard errors; p values are

from repeated-measures mixed-effects models and represent the overall treatment group effect and treatment group $\times$ SECPT time-point interaction. Salivary outcomes were secondary/exploratory and were not adjusted for multiple comparisons

Adverse Reactions and Treatment Discontinuation

No participants reported any serious adverse reactions, and there were no significant group differences in the frequency of self-reported treatment-related adverse reactions. In the placebo condition, 6.7% ($n = 2$) of participants had a treatment-related adverse event. In the ashwagandha group, 3.3% ($n = 1$) of participants reported a treatment-related adverse event (increased irritability). Based on the PGATT completed at visit 3, all participants in

the ashwagandha group who completed the trial reported excellent or good tolerance to the capsules. No participants in the placebo group withdrew from the study; however, one participant in the ashwagandha group withdrew. This was unrelated to the capsule intake (fractured arm).

DISCUSSION

Based on recent systematic reviews and meta-analyses, it has been concluded that

ashwagandha supplementation is more effective than a placebo in reducing self-reported stress and anxiety, and is associated with reductions in serum cortisol [3 4]. However, there has been significant variability in the extracts used, including root-only and combined root-and-leaf preparations. Administered doses have also varied widely, ranging from 250 to 600 mg daily, with treatment periods ranging from 6 to 8 weeks.

The purpose of this study was to examine the anti-stress and anti-fatigue effects of an ashwagandha root extract, Ashwa.30™, in adults experiencing self-reported stress. However, in contrast to previous studies, participants received a significantly lower dose of 30 mg daily and were treated for a shorter treatment duration of 4 weeks. Based on the primary outcome measure (DASS-21 stress score), participants who received ashwagandha experienced a significantly greater reduction in self-reported stress at week 4 than those in the placebo group. This was characterised by a 46.2% reduction in stress score in the ashwagandha group compared to a smaller 32.9% reduction in the placebo group. The PPS analysis was directionally consistent but did not reach statistical significance. This discrepancy may partly reflect the modest sample size and the reduced number of participants included in the PPS. Based on daily stress ratings, participants who received ashwagandha reported lower stress levels during the 4-week study than those who received placebo. From Days 0–28, statistically significant between-group differences were not detected for self-reported anxiety, depression, or fatigue. However, as the study was powered for the primary stress outcome rather than secondary outcomes, these findings should be interpreted as inconclusive rather than as evidence of no treatment effect.

To further examine the anti-stress effects of ashwagandha, participants were exposed to the SECPT, an experimental procedure designed to induce stress, on Days 7 (visit 2) and 28 (visit 3) after intake. On visit 3 (Day 28), group differences in self-reported stress and fatigue ratings, as well as salivary cortisol levels, were identified. This comprised an overall lower stress rating among participants in the ashwagandha group compared to the placebo group, with statistically

significant lower stress ratings immediately after and 10 min after the SECPT. Differences in fatigue trajectories were also observed on Days 7 and 28, with a greater reduction over time in the ashwagandha group compared with the placebo group. However, at both time points, participants in the ashwagandha group reported higher fatigue ratings than those in the placebo group before the SECPT procedure. This baseline difference complicates the interpretation of the observed fatigue trajectories, as greater reductions over time in the ashwagandha group may partly reflect higher pre-stressor fatigue levels and regression-to-the-mean effects rather than a treatment-specific attenuation of stress-induced fatigue. Therefore, the SECPT fatigue findings should be interpreted as exploratory rather than definitive evidence of reduced fatigue reactivity.

On Visit 3 (Day 28), but not Visit 2 (Day 7), salivary cortisol responses differed between groups. Cortisol concentrations remained relatively stable from before the SECPT to 30 min after in the ashwagandha group, whereas they increased in the placebo group. Consequently, cortisol concentrations 30 min after the SECPT were lower in the ashwagandha group than in the placebo group. This finding is best interpreted as possible attenuation of stress-induced cortisol reactivity rather than as evidence of a direct cortisol-lowering effect. The variability in SECPT findings across visits may partly reflect repeated exposure to the stress procedure. During the second SECPT, participants may have experienced reduced novelty, anticipatory stress, or physiological responsiveness. However, because both groups followed the same SECPT schedule, habituation alone is unlikely to fully account for the Day 28 between-group differences in subjective stress ratings and salivary cortisol reactivity. These findings may therefore reflect effects that emerged after a longer duration of Ashwa.30™ supplementation, although a contribution of habituation cannot be excluded. Future studies should use designs that better account for potential habituation effects, such as familiarisation procedures, alternative stress tasks across visits, or non-repeated stress-challenge designs.

No statistically significant between-group differences were detected for most other

physiological stress-response measures, including blood pressure, pulse, skin conductance, or salivary chromogranin A. An isolated group difference in skin temperature was observed at visit 2, with generally higher skin temperature in the ashwagandha group; however, this difference was not replicated at visit 3. Given the modest sample size, exploratory nature of these outcomes, and inconsistent findings across visits and physiological measures, these results should be considered inconclusive and require confirmation in larger studies. Moreover, repeated exposure to the SECPT may have influenced responses at the second visit. Therefore, future studies could use familiarisation procedures, alternative stress tasks, or non-repeated stress-challenge designs to better account for this.

The results from this study provide preliminary evidence that Ashwa.30™ at a dose of 30 mg daily may reduce self-reported stress over a 4-week period in adults aged 18–65 years experiencing self-reported stress. However, as the treatment duration was shorter and the dose substantially lower than in most previous ashwagandha trials, further research is required to determine how these findings compare with higher-dose preparations administered over longer treatment periods. These findings provide preliminary support for possible modulation of the HPA-axis. However, as there were no significant group differences in blood pressure, pulse, temperature, or salivary chromogranin, the influence of these variables on the autonomic nervous system, which provides a more rapid stress/alarm response, could not be identified. Disturbances in the HPA-axis are commonly identified in a range of psychiatric conditions, such as anxiety and depression [25]. A relationship between chronic stress, trauma and HPA-axis disturbances has also been identified [26]. Moreover, HPA-axis imbalances have been identified in medical conditions such as cardiovascular disease, autoimmune conditions, and gastrointestinal diseases [27–29]. While further research is required, the anti-stress and HPA-axis-influencing effects of NRAHG001 identified in this study have potential implications for the supportive management of a range of psychiatric and medical conditions [30].

Directions for Future Research

The overall positive anti-stress effects and good tolerability of Ashwa.30™, as demonstrated in a robust, randomised, double-blind, placebo-controlled study, are encouraging. However, several recommendations are provided for future research. The sample size calculation was based on detecting a moderate-to-large effect on the primary stress outcome. Although this was considered an appropriate pragmatic planning assumption for this signal-detection study, the modest sample size may have limited the ability to detect smaller but potentially clinically relevant between-group effects, particularly for secondary outcomes such as depression, anxiety, fatigue, and physiological stress-response measures. Future studies with larger sample sizes are required to provide more precise estimates of treatment effects and better account for placebo responses in Australian study populations. Treatment expectancy should also be considered when interpreting the self-reported outcomes. Although positive expectancy scores were similar between groups, negative expectancy scores differed at baseline. As expectancy may influence subjective stress, mood, and fatigue ratings, both CTES positive and negative expectancy scores were included as covariates in the revised analyses. However, residual expectancy effects cannot be excluded, and future studies should continue to assess and account for expectancy-related factors when evaluating subjective outcomes.

Participants in the placebo group were older than those in the ashwagandha group at baseline. This imbalance is relevant because age may influence perceived stress and HPA-axis reactivity. Age was therefore included as a covariate in the mixed-effects models. In addition, an exploratory age-by-treatment interaction analysis for the primary Day 28 DASS-stress outcome did not provide evidence that age moderated the treatment effect. However, because this analysis was exploratory and the sample size was modest, this finding should be interpreted cautiously. Larger studies are required to determine whether age modifies the effects of Ashwa.30™ on subjective or physiological stress-response outcomes.

Further studies evaluating the safety, tolerability and efficacy of Ashwa.30™ across different age groups, sexes and clinical populations will be important. Generalisability should also be considered. The present study was conducted in Australia and included a predominantly female sample. However, preliminary evidence from a separate 7-day randomised, double-blind, placebo-controlled study conducted in India in healthy male adults also reported lower post-stressor salivary cortisol concentrations following Ashwa.30™ supplementation compared with placebo during an acute stress challenge [31]. Although this provides supportive evidence across a different population and stress paradigm, the Indian study was short in duration, included only males, and focused mainly on acute cortisol reactivity. Further studies in larger, more diverse populations would be valuable for better understanding the broader applicability of Ashwa.30™ across sex, age, cultural, dietary, lifestyle, and healthcare contexts. Dose-escalation studies may also be important for non-responders to determine whether higher doses are necessary. Moreover, higher doses could be administered at the start of treatment to determine whether symptom alleviation occurs more rapidly. Lower maintenance doses could then be administered to determine if symptomatic improvements are maintained over time. Longer studies will be important to verify the long-term safety and tolerability of this ashwagandha extract, and to determine whether the stress-reducing effects observed after 28 days are sustained over treatment periods more consistent with those of previous ashwagandha trials. Safety monitoring, including measures of liver and renal function, will also be helpful. The effect of Ashwa.30™ on the HPA-axis suggests potential benefits for mental and physical conditions, where HPA-axis dysregulation has been identified. This includes mental health conditions such as depression and anxiety, insomnia, and even medical conditions where stress may play a role, including cardiovascular disease, autoimmune conditions, digestive disorders, and metabolic conditions. Research into other potential mechanisms of action of Ashwa.30™ will also be important. This

includes an examination into its anti-inflammatory, anti-oxidant, neuroprotective, and metabolic effects.

Limitations

Several limitations should be acknowledged. This was a modestly sized signal-detection study, powered for the primary stress outcome, and may therefore have had insufficient power to detect smaller effects on secondary and physiological outcomes. The SECPT was administered twice, and habituation to the procedure may have influenced responses at the second assessment. Baseline differences in age, treatment expectancies, and pre-SECPT fatigue may also have affected some findings, although relevant variables were statistically adjusted where appropriate. In addition, the 28-day treatment duration, predominantly female Australian sample, and exploratory analyses of multiple secondary outcomes without adjustment for multiple comparisons limit the generalisability and certainty of the findings.

CONCLUSIONS

Findings from this randomised, double-blind, placebo-controlled study provide preliminary evidence that 28-day intake of Ashwa.30™ at a dose of 30 mg daily may reduce self-reported stress in adults experiencing perceived stress. This effect was statistically significant in the FAS, with directionally consistent findings observed in the PPS. SECPT findings also suggested lower subjective stress reactivity and attenuation of SECPT-induced salivary cortisol reactivity at Day 28. Although repeated exposure to the SECPT may have influenced responses over time, the significant between-group differences observed at Day 28 suggest that habituation alone is unlikely to account for these findings. Larger trials are required to confirm these findings and determine their clinical relevance and generalisability.

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

Declarations

Conflict of interest. Adrian Lopresti is the Managing Director of Clinical Research Australia, a contract research organisation that has received research funding from nutraceutical companies. Adrian Lopresti has also received presentation honoraria from nutraceutical companies. Stephen Smith is an employee of Clinical Research Australia and declares no other conflicts of interest. Deepak Mundkinajeddu, Prashanth D'Souza, Suresh Lakshmiathan, Bharathi Bethapudi and Auxi Arobana R are employees of Natural Remedies Pvt Ltd., the funder of the study. The funder was not involved in data collection, data interpretation or the decision to submit the manuscript for publication.

Ethical Approval. The trial was conducted in compliance with the study protocol, Good Clinical Practice and applicable regulatory requirements. The study commenced only after a favourable ethical opinion was obtained from the National Institute of Integrative Medicine-Human Research Ethics Committee (approval number: 0153E_2025). Written informed consent was obtained from all participants before commencement of the study.

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