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Original article

Clinical Validation of the Potential of Herbal Extract Blend in Alleviating the Stress and Related Conditions: A Randomized, Placebo-Controlled, Cross-Over Clinical Study

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Abstract

Background: The global rise in stress-related conditions has increased the demand for effective and safe interventions. This study aims to validate the efficacy of BacoZen™ blend tablets, an herbal blend of Ashwagandha extract and Brahmi extract, in alleviating stress. ***Methodology:*** A randomized, placebo-controlled, cross-over clinical trial was conducted with 64 subjects. Group A received BacoZen™ blend tablets for 4 weeks (Part I), followed by a 1-week washout period and placebo for 4 weeks (Part II). Group B received the interventions in reverse order. Assessments included perceived stress, cortisol, dopamine, sleep parameters, mood, fatigue, anxiety, and withdrawal symptoms. ***Results:*** The test group exhibited significant reductions in perceived stress (31.78%, 32.46%), cortisol levels, and improvements in dopamine levels, sleep quality, mood, fatigue and anxiety compared to the placebo group in both treatment parts. No adverse events or clinically significant changes in vital signs were reported, indicating excellent tolerability. The crossover design confirmed the intervention's consistent efficacy, independent of the sequence. ***Conclusion:*** BacoZen™ blend tablets containing herbal extracts Ashwagandha and Brahmi demonstrated robust and reproducible therapeutic effects in managing stress and anxiety, with an excellent safety profile. These promising results substantiate its potential as a safe and effective alternative to conventional interventions.

Keywords: ***Stress, Anxiety, Fatigue Withania somnifera, Bacopa monnieri, Cortisol.***

Introduction

The prevalence of stress-related conditions is rising globally [1]. Prolonged stress can cause mental and physical issues, including depression, high blood pressure, and metabolic disorders. During the COVID-19 pandemic, estimates revealed that 28% experienced depression, 26.9% had anxiety, 24.1% had post-traumatic stress disorder (PTSD) symptoms, 36.5% were stressed, 50% faced psychological distress, and 27.6% had sleep problems [2].

Trauma or life stressors can precede or trigger stress-related psychiatric conditions like PTSD, acute stress disorder, and other stress disorders [3]. Several disorders are linked to stress system dysfunction, including obesity, type 2 diabetes, hypertension, autoimmunity, allergies, anxiety, insomnia, depression, pain, and fatigue [4,5]. An unhealthy diet, lack of exercise, substance abuse, and sleep deprivation exacerbate daily challenges. Low self-esteem individuals struggle with trauma processing and are prone to stress-induced health conditions [6].

Dopamine and cortisol serve as crucial stress biomarkers. While dopamine increases during acute stress, particularly in cases of strong fear or anxiety, cortisol exhibits a more complex response. Cortisol levels peak 20-30 minutes' post-stressor and show attenuated HPA axis response in chronic stress, making it valuable for assessing long-term stress in patients [7].

Efforts to manage stress are shifting towards alternatives to benzodiazepines due to their adverse effects and withdrawal issues. Traditional herbal remedies, known for their long history of use, are gaining interest as potential treatments, though they have seen limited scientific study [8].

Traditional medicine uses various herbs to manage stress, anxiety, fatigue, and insomnia. Adaptogens, like *Withania somnifera* and *Bacopa monnieri*, are plant-based substances that help the body

resist stress and restore normal functions. These herbs are known for their neuroprotective, anti-fatigue, antidepressant, and anxiolytic properties [9]. Ashwagandha has shown positive effects in reducing stress and anxiety and improving memory and cognition [10]. Brahmi is recommended for mental stress, memory loss, epilepsy, insomnia, and cognitive enhancement [11]. This study aims to validate the potential of a Proprietary BacoZen™ in alleviating stress and related conditions.

Materials and methods

Study design

We conducted a randomized, placebo-controlled, cross-over clinical trial to assess the efficacy of an BacoZen™ in alleviating stress. BacoZen™ is coded as NBA-23 for the clinical trial. Sixty-four participants were divided into two groups, each with 32 subjects. Group A received the BacoZen™ for 4 weeks, while Group B received a placebo. After a 1-week wash-out, treatments were reversed. The study analyzed data from 128 participants over 70 days, with efficacy assessments on specific days and measurements of cortisol, dopamine, and sleep parameters. Subjects were advised to take 1 tablet twice a day, with an interval of 11-14 hours, after food. Subjects were advised to not take the tablet empty stomach. The trial was approved by the Institutional Ethics Committee of Lokmanya Medical Research Centre, Pune, and registered with the Clinical Trials Registry - India (CTRI/2023/09/057801). The consolidated standards of reporting trials (CONSORT) flow of the entire study is depicted in Figure 1.

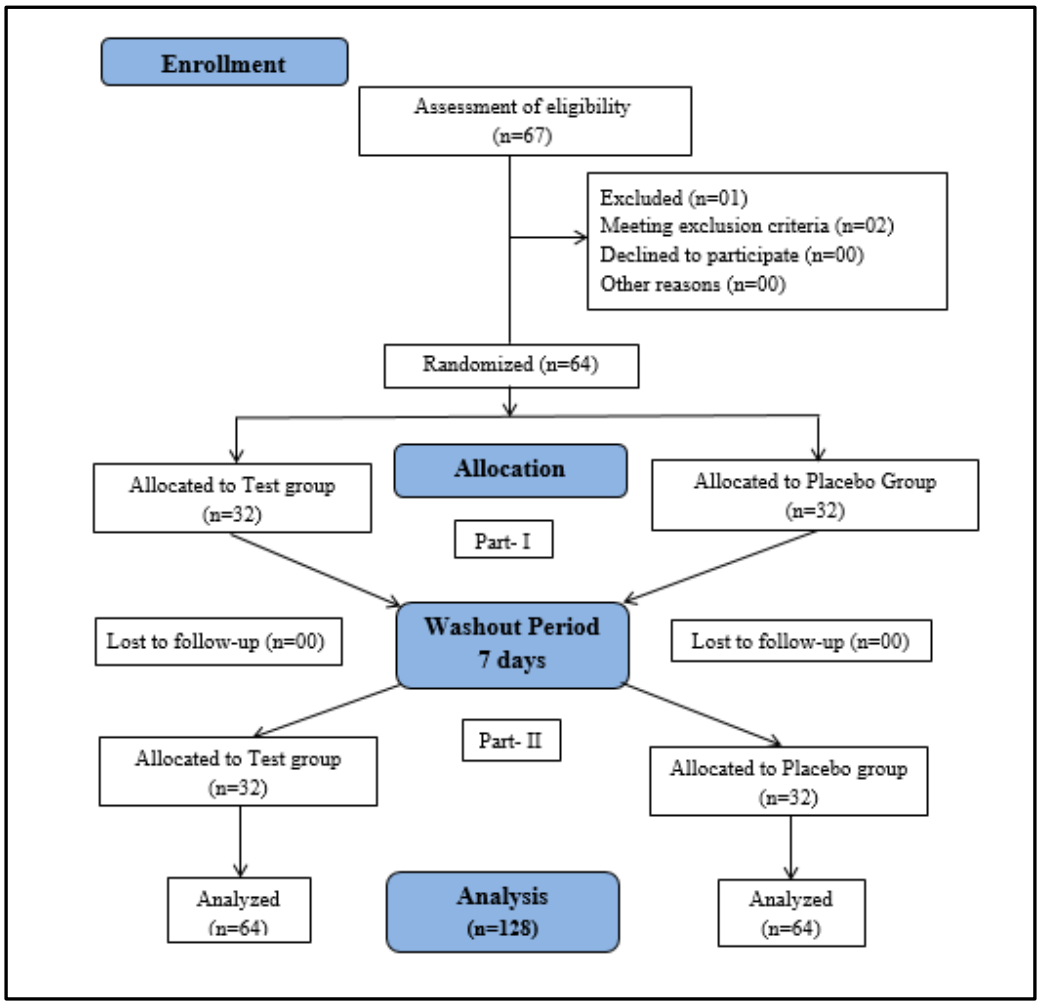

Figure 1: CONSORT flow diagram

Inclusion Criteria

Subjects aged 30 to 59 years, diagnosed with primary insomnia per DSM-V-TR criteria, with an Insomnia Severity Index score between 8 and 20 (mild to moderate), and a Perceived Stress Scale (PSS) score of 14-26 were included. Female subjects of reproductive potential with a negative pregnancy test who agreed to use contraception during the study were eligible. Participants had to be willing to sign the informed consent form, limit alcohol, caffeine, and nicotine consumption, and be free of psychiatric conditions other than perceived stress.

Exclusion Criteria

Subjects were excluded if they had difficulty sleeping due to a medical condition, a history of neurological disorders, bipolar disorder, psychotic disorder, posttraumatic stress disorder, or any current psychiatric disorder requiring medication. Individuals with ongoing clinical depression or anxiety disorder (moderate to severe diagnosis on PHQ-9 and GAD-7 scales), history of substance abuse or dependence, or evidence of a clinically significant cardiovascular disorder at the pre-study visit were excluded. Additionally, those taking prohibited medications, consuming more than 15 cigarettes per day, or having a history of malignancy within five years prior to consent were also excluded. Any condition deemed by the investigators to make the subject unsuitable for enrollment or that could interfere with participation and completion of the protocol also led to exclusion.

Methodology

This was a randomized, placebo-controlled, cross-over clinical trial validating potential of BacoZen™ in alleviating the stress and related conditions. On the screening visit, written informed consent

was obtained from the subjects for their participation in the study. Subject's demographics, medical, surgical, treatment history, and current medication if any were recorded. Efficacy assessments was done on each visit (baseline/day 1, day 28, day 36, and day 63) to assess clinical improvement, vitals, symptoms and rescue medication. Concomitant diseases & medication assessment was done on screening and baseline. Assessment of cortisol and dopamine levels at screening, day 28, and day 63. Subject -reported time to sleep onset (as per subject diary), changes in sleep latency, sleep efficiency (Total sleep time/ time in bed*100), and subject -reported number of awakenings derived from the subject diary was assessed at day 28, day 36, and day 63. Assessment of perceived stress scale (PSS) score by perceived stress questionnaire, severity of insomnia using the Insomnia Severity Index Scale and daytime fatigue using Fatigue Severity Scale (FSS) was assessed from screening to day 63. Assessment of measurement of mood on the POMS scale (Profile of mood state questionnaire) and changes in anxiety by using Hamilton Anxiety Rating Scale (HAM-A) score was assessed from screening to day 63. Assessment of habit forming- withdrawal symptoms after stopping treatment for 1 week at day 70 (week 10 telephonic follow-up). Treatment compliance, safety and tolerability of the study intervention in terms of adverse events (AEs), were assessed from baseline to end of the study. All the scales and questionnaires used in the study are validated and widely accepted.

Investigational Product Composition

Composition along with the content details is expressed in Table 1.

Table 1: Active ingredients of BacoZen™

Sr. No.	Name of the Ingredient	Scientific Name	Composition
1.	Brahmi Extract (Aerial parts)	*Bacopa monnieri*	143 mg
2.	Ashwagandha Extract (Roots)	*Withania somnifera*	100 mg

Statistical analysis

Statistical analysis has been done by using SPSS software. The primary and secondary endpoints were analyzed using the dependent and independent Student t-test, Chi-square test, and Mann-Whitney U test. The p-values ≤0.05 were considered statistically significant.

Results

Assessment of Demographic Characteristics

The treatment and placebo groups were well-balanced in terms of age (42.41 ± 8.51 years) and gender distribution (11 males and 21 females in each group), with no significant differences observed.

Assessment of Perceived Stress Scale Score (PSS)

The test group exhibited a significantly greater reduction in PSS scores compared to the placebo group in both Part I (31.78% vs. 16.06%) and Part II (32.46% vs. 14.75%), indicating the consistent and significant efficacy of the test treatment over placebo in reducing perceived stress through the crossover study design as shown in Table 2.

In Part I, the test group had a 35.64% reduction in Fatigue Severity Score (FSS), significantly greater than the 15.89% reduction in the placebo group. In Part II, the test group saw a 31.63% reduction, while the placebo group had a 20.52% reduction. The consistent FSS reduction in the test group across both parts confirms its significant efficacy over placebo.

Table 2: Assessment of changes in perceived stress score and fatigue severity score between groups

Perceived Stress Scale Score (PSS) (Mean ± S.D)			
Duration	**Test (n = 32)**	**Placebo (n = 32)**	**P value (Between group)**
Treatment Part I			
Screening	20.06±3.36	19.84±3.44	0.798
Day 28	13.69±3.80*	16.66±3.53*	< 0.01
Treatment Part II			
Day 36	17.81±2.52	15.25±3.86	< 0.01
Day 63	12.03±3.33*	13.00±3.85*	< 0.01
Fatigue Severity Score (Mean ± SD)			
Treatment Part I			
Screening	3.46±0.67	3.34±0.63	0.481
Day 28	2.23±0.72*	2.81±0.50*	< 0.01
Treatment Part II			
Day 36	2.92±0.43	2.39±0.54	< 0.01
Day 63	2.00±0.43*	1.90±0.56*	< 0.01

*Data is represented as Mean ± S.D. Between group analysis was done using an independent student t-test. Within group analysis was done using dependent student t-test. Significant at p< 0.05 * indicates within group significant p value.*

Assessment of Serum Cortisol and Dopamine Levels

In Part I, after 28 days of treatment, the test group experienced a substantial 21.25% decrease in serum cortisol, while the placebo group showed a 15.26% increase. Part II revealed an additional 28.66% reduction in the test group, contrasting with a 16.37% rise in the placebo group. These results demonstrate that the test treatment consistently lowered serum cortisol levels more effectively than the placebo.

Serum dopamine levels were similar at screening. After 28 days in Part I, both groups had increased dopamine, with the test group improving by 17.87% versus 10.34% for placebo. In Part II, the test group showed a 34.52% increase, while the placebo group decreased by 9.1%. The test treatment resulted in significantly higher dopamine levels compared to placebo in Part II as shown in Table 3.

Table 3: Assessment of changes in serum cortisol and serum dopamine levels between groups

Serum Cortisol Level (Mean ± SD) (ng/mL)			
Duration	**Test (n=32)**	**Placebo (n=32)**	**P value (Between group)**
Treatment Part I			
Screening	111.74±27.16	90.72±55.33	0.06
Day 28	88.00±48.47*	104.57±42.97	0.013
Treatment Part II			
Duration	**Test (n=26)**	**Placebo (n=32)**	**P value**
Day 63	74.60±41.02*	102.40±38.10	0.005
Serum Dopamine Level (Mean ± SD) (mg/dL)			
Treatment Part I			
Screening	5.46±1.38	4.50±1.42	0.008
Day 28	6.44±1.42*	4.96±1.47*	0.039
Treatment Part II			
Day 63	6.68±1.29*	5.85±1.31*	< 0.01

*All data was analyzed by student T-Test Independent for between group and by student T-Test Dependent for within the group. Significant at p< 0.05. * indicates within group significant p value.*

Assessment of Sleep Diary

The test group showed consistent results between Part I and II, with improved total sleep time, efficiency, and reduced sleep latency, awakenings, and sleep onset time. This indicates that the test product enhances sleep patterns in both treatment phases Table 4.

Table 4: Assessment of sleep diary

Assessment of Sleep Diary (Mean ± SD)				
Treatment Part I				
Parameters	**Duration**	**Test**	**Placebo**	**P Value (Between group)**
Total Sleep Time (min)	Day 1	300.47±40.77	298.28±35.48	0.833
	Day 28	353.32 ± 31.66*	316.55 ± 35.84*	< 0.01
Sleep Latency (Time to Sleep Onset) (min)	Day 1	97.50±18.67	99.38±14.13	0.659
	Day 28	80.04 ±11.41*	89.18 ± 10.60*	0.009
Sleep efficiency (%)	Day 1	66.40±5.71	66.16±4.50	0.490
	Day 28	76.75 ± 4.30*	72.41 ± 4.37*	< 0.01
Subject Reported no. of Awakenings (numbers)	Day 1	2.69±0.74	3.00±0.92	0.167
	Day 28	1.83 ± 0.86*	2.08 ± 0.83*	0.035
Subject reported wake time after sleep onset (numbers)	Day 1	52.97±13.73	52.34±13.97	0.849
	Day 28	26.51 ± 12.53*	30.65 ± 12.28*	0.503
Treatment Part II				
Total Sleep Time (min)	Day 36	299.06±36.93	288.75±30.24	0.395
	Day 63	356.81 ± 23.14*	312.44 ± 32.68*	< 0.01
Sleep Latency (Time to Sleep Onset) (min)	Day 36	96.56±14.73	96.56±12.60	0.968
	Day 63	79.15 ± 6.24*	85.04 ± 10.25*	0.484
Sleep efficiency (%)	Day 36	65.72±4.50	64.73±2.72	0.133
	Day 63	77.05 ± 2.28*	72.26 ± 3.63*	< 0.01
Subject Reported no. of Awakenings (numbers)	Day 36	2.69±0.59	3.03±0.90	0.141
	Day 63	1.79 ± 0.67*	2.30 ± 0.74*	0.002
Subject reported wake time after sleep onset (numbers)	Day 36	58.13±7.38	60.00±0.00	0.155
	Day 63	26.98 ± 10.20*	34.43 ± 10.14*	0.054

*Data is represented as Mean ± S.D. All data was analyzed by student T-Test dependent means and Wilcoxon Signed-Ranks Test for within the group. Significant at p< 0.05. Between-group assessment is performed on the difference mean of either day 1 and day 28, day 36 and day 63 respectively. * indicates within group significant p value.*

Assessment of Changes in Insomnia Severity Index

At screening, both groups had similar subthreshold insomnia with comparable ISI scores. After 28 days, the test group showed a significant 43.51% reduction in ISI score, compared to 16.34% for the placebo group. In Part II, by day 63, the test group reduced ISI scores by 43.77%, while the placebo group showed a 22.48% reduction Table 5.

Table 5: Comparison of changes in the insomnia severity index score between the groups

Insomnia Severity Index Score (Mean ± SD)			
Duration	**Test (n = 32)**	**Placebo (n = 32)**	**P value (Between group)**
Treatment Part I			
Screening	13.72±3.15	12.81±1.82	0.164
Day 28	7.75±2.71*	10.72±1.71*	< 0.01

% Change	43.51	16.34	
Treatment Part II			
Day 36	11.28±1.22	9.59±2.60	0.002
Day 63	6.34±1.04*	7.44±2.688	< 0.01
% Change	43.77	22.48	

*All data was analyzed by student T-Test Independent for between groups and by student T-Test Dependent for within the group. Significant at p< 0.05. * indicates within group significant p value.*

Assessment of POMS scale score

At screening, both groups had similar good mood scores. In Part I, the test group saw a significant 138% increase in good mood, compared to 64% for the placebo group. In Part II, the test group improved by 35%, while the placebo group's score decreased by 45%.

Bad mood scores were also similar at screening. In Part I, the test group had a significant 76% reduction, versus 45% for the placebo group. In Part II, the test group maintained a 65% reduction, while the placebo group had a non-significant 14% increase. The test intervention significantly improved mood across both phases of study.

Assessment of Hamilton Anxiety Rating Scale Score

When compared at the end of the part II study, the reduction in HAM-A score was comparable for the test group in both parts (35.32 % reduction in part I and 38.15% reduction in part II). This indicates that the treatment with the test is showing a similar pattern of HAM-A score reduction in both parts, confirming its activity through crossover.

Assessment of modified sleep regularity and medication withdrawal questionnaire

The test group showed significantly better sleep regularity (mean score: 19.69±1.93) compared to the placebo group (mean score: 9.94±1.66). After treatment discontinuation, both groups experienced mild withdrawal symptoms, such as confusion and mood changes, which resolved by day 70 without medical intervention

Discussion

The study evaluated the effectiveness of BacoZen™ blend tablets in stress relief using a randomized, placebo-controlled cross-over trial. In Part I, the test group received a BacoZen™ blend, and the control group received a placebo, followed by a 7-day washout [12,13].

The test group experienced significant reductions in perceived stress (31.78% in Part I and 32.46% in Part II) compared to the placebo group. Cortisol levels decreased, and dopamine levels increased more in the test group. Improvements in sleep metrics and fatigue were notable, with a 35.64% reduction in Part I and 31.63% in Part II for fatigue. Mood improved significantly in the test group with a 138% increase in Part I and 35% in Part II, while anxiety decreased more in the test group. Post-treatment assessments showed good sleep regularity, no addictive properties, excellent tolerance, and stable vital signs, with 100% compliance and no adverse events.

The study acknowledges the role of Ayurvedic herbs Brahmi (*Bacopa monnieri*) and Ashwagandha (*Withania somnifera*) in mental health management. Brahmi is traditionally used in Ayurveda for treating *Chittodwega*, a psychiatric disorder attributed to imbalances in Manasika dosha, owing to its stress-relieving, anxiolytic, and antioxidant properties [14]. Brahmi has shown efficacy in mitigating anxiety, depression, and stress during the COVID-19 pandemic [15] and reducing stress-induced cortisol release in zebrafish larvae [16], supporting its traditional use as a neural tonic and aligning with the positive outcomes observed in the current trial. Ashwagandha has demonstrated significant reductions in anxiety levels and improvements in depression, anxiety, and stress scores, similar to our study's results [17]. Ashwagandha benefits include moderating HPA axis activity by reducing morning cortisol and DHEA-S levels. Its anxiolytic effects are due to antioxidant and anti-inflammatory properties, which alleviate inflammation and oxidative stress linked to stress, depression, and anxiety [18].

Preclinical studies suggest Ashwagandha affects GABAergic and serotonin activity, enhancing mood. Long-term use improves memory, attention, sleep, and psychological well-being, reducing stress and showing good safety and tolerability. The active compound, ferulate doconasil, provides anxiolytic effects similar to diazepam but without its side effects [19].

Bacopa monnieri supplementation prevents dopamine depletion due to stress, suggesting adaptogenic and protective effects on dopaminergic neurons [20,21]. These findings, consistent with our study, indicate that Brahmi and Ashwagandha in the formulation may contribute to dopamine regulation and positive outcomes in stress-related conditions. Furthermore, no side effects were observed during treatment, and any reported symptoms resolved without medication, indicating the intervention's safety and non-addictive nature for stress management.

The study's randomized, placebo-controlled, crossover, double-blind design, with 28-day treatment phases and each subject as their own control, strengthened its findings. The test group showed significant mean cortisol reductions of 23 ng/ml in Part I and 31 ng/ml in Part II, indicating a role in circadian rhythm regulation [22]. Future research should determine the washout period through pharmacokinetic studies of the test product.

Conclusion

The test group consistently demonstrated significant improvements in perceived stress, serum dopamine levels, sleep parameters, insomnia severity, fatigue, mood states, and anxiety compared to the placebo group before and after crossover. Furthermore, no clinically significant adverse events or withdrawal symptoms were reported, highlighting the excellent tolerability and safety profile of BacoZen™ blend tablets. The crossover design reinforces the reliability of the findings, as the positive outcomes were independent of the intervention sequence. These promising results substantiate the potential of this herbal formulation as a versatile and efficacious solution for managing stress and its associated consequences, offering a safe and natural alternative to conventional pharmacological interventions.

Declarations

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Data Availability

The datasets generated and/or analysed during the current study are not publicly available due to Intellectual property constraints but are available from the corresponding author on reasonable request.

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