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A double-blind, randomized, placebo-controlled study on the efficacy of standardized processed Ashwagandha root powder in alleviating stress and anxiety with reference to sleep quality, testosterone, and hGH levels

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Chronic stress sets off a complicated neuroendocrine reaction that includes changes in anabolic hormones like testosterone and human growth hormone (hGH), sleep difficulties, and psychiatric disorders. Comprehensive clinical benefits may be provided by therapeutic strategies that can address these interrelated physiological and psychological alterations. Ashwagandha (*Withania somnifera*), is a well-known adaptogenic plant with endocrine-regulating and stress-relieving qualities. The purpose of the study was to assess how standardized processed Ashwagandha root powder affected mild to moderate stress and anxiety in persons between the ages of 18 and 45. These 104 individuals were divided into two groups at random and given an standardized processed Ashwagandha root powder cap (300 mg twice daily) in Group A and a placebo cap for 30 days in Group B. The Pittsburgh Sleep Quality Index (PSQI), Depression Anxiety Stress Scale (DASS), and Perceived Stress Scale (PSS) were used to evaluate the results. Prior to and following the intervention period, serum levels of hGH and testosterone were assessed. When compared to the placebo group, participants who received standardized processed Ashwagandha root powder showed notable improvements in sleep quality as indicated by PSQI scores ($p<0.001$) and significant decreases in stress and anxiety scores on both the PSS and DASS scales ($p<0.001$). Additionally, after supplementation, there were notable increases in serum levels of hGH and testosterone ($p<0.01$). Nevertheless, during the trial period, no adverse events were reported. These results suggest that standardized processed Ashwagandha root powder may have positive effects on hormonal, psychological, and sleep-related parameters linked to stress-induced neuroendocrine imbalance.

Keywords: Adaptogens, Anxiety, Psychological stress, Randomized controlled trial, Testosterone, *Withania somnifera*

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In today's society, stress and anxiety are a major health concern, and are strongly linked to disturbances in neuroendocrine regulation, disturbed sleep patterns and hormonal alterations including reduced secretion of testosterone and human growth hormone (hGH). Chronic psychological stress triggers activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system leading to increased cortisol production, suppression of reproductive hormones and perturbation of normal sleep physiology. These neurohormonal disturbances have an adverse effect on emotional well-being, cognitive performance, physical energy, sexual health, and overall quality of life[1-4]. Conventional pharmacological therapies are widely used to manage anxiety and sleep-related disorders; however, their long-term use is often associated with adverse effects including sedation, dependence, and decreased long-term compliance[5]. As a result, scientific interest in herbal and natural interventions that can safely improve stress resilience and restore physiological balance is increasing. *Withania somnifera* (Ashwagandha), a prominent Rasayana drug described in Ayurveda, has been traditionally used to enhance vitality, promote mental stability, and improve adaptation to stress[6]. Recent experimental and clinical studies have shown adaptogenic, anxiolytic, neuroprotective and endocrine-regulatory activities of Ashwagandha. These effects are mainly attributed to its bioactive compounds including withanolides and sitioindosides, which are

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thought to modulate stress-mediating pathways and neurotransmitter regulation[7,8]. Previous clinical investigations have reported beneficial effects of Ashwagandha in reducing stress and anxiety through modulation of cortisol secretion and GABAergic signalling pathways[9,10]. Emerging evidences show its potential role in improving sleep quality and increasing serum testosterone levels[11].

Therefore, the present study was designed to evaluate the efficacy and safety of standardized processed Ashwagandha root powder (aqueous extract) in individuals experiencing stress and anxiety with special reference on perceived stress, anxiety levels, sleep quality, serum testosterone and hGH concentrations. The study was designed as double-blind, randomized, placebo-controlled clinical design to ensure methodological reliability and minimize bias.

The main aim of the study was to assess the efficacy of standardized processed Ashwagandha root powder for reduction of stress and anxiety in adults.

Secondary objectives were to evaluate its efficacy in reducing stress and anxiety by both subjective (questionnaire-based) and objective (biochemical) parameters, to evaluate its effect on sleep quality by validated sleep assessment tools and to study its effect on serum testosterone and human growth hormone (hGH) levels.

Materials and Methods

Study design

The present study was a single-centre, prospective, double-blind, randomised, placebo-controlled clinical trial done to evaluate the efficacy and safety of standardized processed Ashwagandha root powder in reducing stress and anxiety in adults.

Eligibility criteria

Inclusion criteria

Participants should have a score of at least 14 on the Perceived Stress Scale (PSS), be between the ages of 18 and 45 years (inclusive), be male or female, and have no history or diagnosis of psychiatric problems.

Exclusion criteria

Participants with a score of less than 14 on the Perceived Stress Scale (PSS) at the time of screening, less than 18 years old or more than 45 years old (regardless of gender), with a previous diagnosis of a mental disorder or who was receiving ongoing mental therapy were excluded from the study.

Discontinuation criteria

During the study, any participant experienced a serious illness which required emergency medical attention or a health condition deemed unacceptable for continuing trial participation, they were removed from the research. Additionally, participants were withdrawn at any point during the study period if they voluntarily decided to stop or revoked their informed consent.

Sample size calculation

The sample size was calculated based on the change in Perceived Stress Scale (PSS) scores as the primary outcome measure. The anticipated effect of treatment was determined based on previous clinical trials assessing the stress-alleviating properties of *Withania somnifera*. For the calculation, an effect size (Cohen's d) of 0.6 was assumed. Using a two-tailed statistical test, with a significance level (α) of 0.05 and statistical power of 80% ($1-\beta$), the minimum sample size required was calculated to be 45 individuals in each study arm. The sample size was raised to 56 participants per group to allow for an attrition rate of around 20%. The study enrolled a total of 112 people.

Randomization, allocation concealment, and blinding

A total of 112 eligible participants were randomized at a 1:1 ratio into two groups. Randomization was performed using a computer-generated block randomization method, with a fixed block size of four to ensure even distribution between the intervention and placebo groups. The Sequentially Numbered, Opaque, Sealed Envelope (SNOSE) method was used to maintain allocation concealment. A separate investigator, who was not involved in participant recruitment, intervention delivery, or outcome assessment, prepared and coded the envelopes. The study used a double-blind procedure, which means that participants and evaluators were unaware of which group they belonged to at all times during the study. The research drug and placebo were both manufactured as identical pills with similar appearance, packaging and labeling in order to maintain blinding.

Seven were lost to follow-up because of poor compliance or inability to attend scheduled assessments and one person voluntarily withdrew consent after initiation of the intervention. So, 104 participants' data were included in the final statistical analysis with 52 people in each group.

Interventions

Final analysis was conducted on 104 individuals who met the study protocol and were randomly assigned to 2 groups in equal numbers by utilizing a block randomization procedure to ensure equitable distribution. Participants in Group A (Ashwagandha group, n=52) were given capsules of 300 mg standardized full spectrum processed Ashwagandha root powder twice day with water after meals for 30 days. Participants in group B (placebo group, n = 52) took visually identical placebo capsules containing an inert chemical for the same duration and dose schedule. The trial was conducted in a double-blind manner. The dose schedule of standardized processed Ashwagandha root powder (300 mg twice a day; total daily dose 600 mg) was established based on data from past clinical studies showing positive effects in reducing stress, stress-related parameters, anxiety, sleep and hormonal control. Previous randomized controlled studies have demonstrated that standardized processed Ashwagandha root powder in the range of 300-600 mg per day are safe and beneficial for use in adults.

Moreover, the dose selected was within the usual therapeutic range for supplementation with Ashwagandha and has been well tolerated in human subjects. The rationale for the administration of two divided dosages is to aid in maintained physiological activity and boost the overall therapeutic response over the entire period of intervention.

Outcomes

Primary outcome measures

The primary outcome measures were changes in validated subjective assessment methods for psychological well-being and sleep quality. Perceived stress levels were measured using the Perceived Stress Scale (PSS) while emotional characteristics like depression, anxiety and stress were examined using the Depression Anxiety Stress Scale (DASS-21). Sleep quality in the last month was evaluated using Pittsburgh Sleep Quality Index (PSQI).

Secondary outcome measures

Secondary outcome assessments were objective biochemical markers related to endocrine function. These included blood total testosterone, free testosterone and human growth hormone (hGH) levels and were tested to evaluate the hormonal effects of the intervention.

Safety assessment

Any adverse event reported by the participants was noted in the adverse event reporting protocol. The record included the name of the participant, nature of the adverse event, date of occurrence, severity, relatedness to the intervention and the treatment delivered.

Participant timeline

Participants were enrolled into the study on Day 1 after screening and eligibility were confirmed according to the pre-determined inclusion and exclusion criteria. Demographic details, medical history, clinical examination findings and disease specific observations were recorded on structured case record forms as baseline data. The initial evaluation involved recognized subjective measures such the Pittsburgh Sleep Quality Index (PSQI), Depression Anxiety Stress Scale (DASS) and Perceived Stress Scale (PSS). Baseline biochemical analyses included serum levels of human growth hormone (hGH), free testosterone and total testosterone.

During the intervention course, participants were observed every two weeks. At each follow-up visit, subjective outcome measures, such as PSS, DASS, and PSQI scores, were re-evaluated to determine ongoing clinical changes. On the 30^{th} and 60^{th} days, a comprehensive re-examination was performed, which comprised the objective biochemical markers and subjective ratings. These follow-up examinations had the aim to determine the immediate and long-term therapeutic effects of the intervention.

Data collection methods

The study-related data were collected and maintained in a systematic manner in paper Case Report Forms (CRFs) and electronic Case Report Forms (e-CRFs). The use of both paper-based and digital documentation in combination helped to ensure that the data recorded were correct, consistent, and complete, in addition to lessening the chance of transcription errors. All study documentation and data management protocols were according to the International Council for Harmonization (ICH) guidelines and Good Clinical Practice (GCP) standards. CRFs were cross-checked with the original source papers periodically to guarantee data authenticity and traceability. Independent clinical monitoring was periodically conducted to assure the overall quality of data, to check the accuracy of recorded data, and to ensure compliance with the study protocol. The confidentiality of participants was rigorously

safeguarded in the course of the investigation. For the purposes of anonymity, CRFs and e-CRFs were coded with unique study codes in place of any personal identification. Access to the study records was limited to approved research personnel. Electronic documents were stored in a secure password secured system with audit trail capabilities to track any changes or additions made during the course of the investigation.

Data were archived and stored according to institutional and regulatory obligations. Electronic data was stored on encrypted back-up systems to prevent unintended loss or inappropriate access. Physical CRFs were stored in secure, secured storage within the study site. Regular quality assurance assessments were also performed to maintain the scientific integrity of the study results and ensure compliance with regulatory requirements.

Ethics

The study protocol was accepted by the Institutional Ethics Committee (IEC) of Post Graduate Institute of Ayurved, Dr. Sarvepalli Radhakrishnan Rajasthan Ayurved University (DSRRAU), Jodhpur on August 31, 2024 with clearance number DSRRAU/PGIA/IEC/24-25/737. The clinical trial was prospectively filed with the Clinical Trials Registry–India (CTRI) under registration no. CTRI/2024/09/074425. The study was carried out in accordance with the ethical principles of the Declaration of Helsinki and the recommendations of the Indian Council of Medical Research (ICMR).

Statistical analysis

Statistical analysis was done using appropriate statistical software (e.g., SPSS, version XX.X). Quantitative variables were reported as mean ± standard deviation (SD) for normally distributed data and median (interquartile range [IQR]) for non-normally distributed data. Qualitative variables were expressed as frequencies and percentages.

The Shapiro-Wilk test was employed to assess the normality of the data. The Wilcoxon signed-rank test was employed for skewed or non-parametric data for intragroup comparisons and paired t-tests for variables with normal distribution. The groups were compared using the independent samples t test for parametric data and the Mann-Whitney U test for non-parametric data.

Bonferroni adjustment was applied when appropriate, to limit type I error due to repeated comparisons between primary and secondary outcome variables. Two-sided p values less than 0.05 were considered to be statistically significant after adjustment.

Result

In this clinical study, initially, 112 patients of stress and anxiety were enrolled to evaluate the therapeutic efficacy of processed ashwagandha root powder. During the monitoring period one participant was lost for several reasons such as non-compliance, withdrawal of consent or personal inconvenience. Additionally, seven individuals did not complete the follow-up phase. This may have been due to moving, lack of interest, or difficulty making follow-up sessions. A total of 104 subjects completed the whole study protocol and were included in the final statistical analysis. The recruitment, allocation, follow-up, and analysis of participants are summarized in the CONSORT flow diagram (Fig. 1). In clinical research including psychological and behavioral aspects, where adherence and follow-up in the long term are sometimes challenging, the overall dropout rate of 7.14% may be considered acceptable. However, the final sample size was sufficient to allow for relevant statistical analyses of both primary and secondary end measures. The high completion rate further demonstrates satisfactory patient acceptance, tolerance and compliance with trial intervention. Analysis of demographic data showed that the highest percentage of participants was in the age range of 21 to 30 years old (43.27%). The bulk of the participants (61.54%) were women and 57.69% of the subjects were married. The bulk of the participants were students (41.35%). Most of the respondents (72.12%) were from the Jangala region. Regarding the socioeconomic status, most (82.69%) were from the middle class. Vegetarian dietary habits were observed in 75% of the study subjects. Sama Agni was detected in 54.81% of cases and Madhyama Kostha in 69.23% of cases in the Ayurvedic criterion examination. The educational status showed that 39.42% of individuals have degree. Half the people in the research indicated they had never developed an addiction. The most often noticed was Twak Sara which constituted 25% of participants. The bulk of the participants showed Madhyama Samhanana (85.58%), Madhyama Pramana (86.54%), Sarvarasa Satmya (84.62%) and Madhyama Satva (78.85%). Similarly, majority of the study subjects showed Madhyama Abhyavaharana Shakti (76.92%), Madhyama Jarana Shakti (77.88%) and Madhyama Vyayama Shakti (70.19%). All the contestants were of

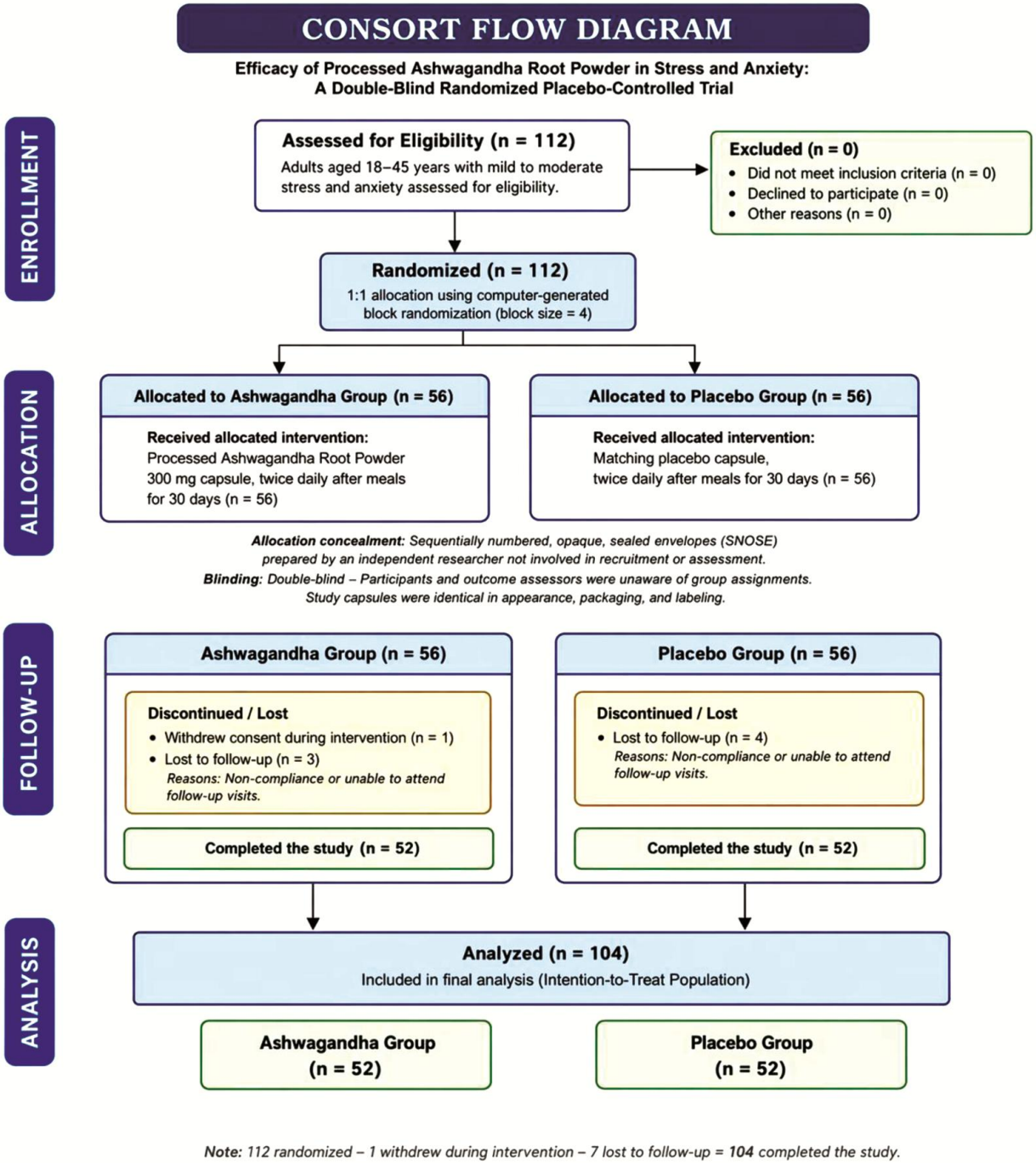


Fig. 1 — CONSORT flow diagram

Madhyama Vaya category. Among the different constitutional types, Vata-Pittaja Prakriti was the most common (76.92% of the subjects).

The present investigation assessed the efficacy of standardized processed Ashwagandha root powder on key subjective domains—stress, anxiety, fatigue, and sleep quality—in Group A. The Depression Anxiety Stress Scale (DASS) showed a substantial reduction in mean scores from 41.20 (baseline) to 12.38 (post-intervention), amounting to a 69.95% decrease. Similarly, the Fatigue Assessment Scale (FAS) reflected a 69.48% reduction, highlighting the adaptogenic and anti-fatigue effects attributed to Ashwagandha in traditional literature and recent trials. The Perceived Stress Scale (PSS) demonstrated a 69.37% reduction in stress perception, while sleep

quality improved by 68.51% as measured by the Pittsburgh Sleep Quality Index (PSQI). The Wilcoxon Signed-Rank Test yielded highly significant results ($p < 0.0001$) across all subjective parameters. These outcomes not only confirm statistical significance but also reinforce clinical relevance. Group B, which received placebo capsules, showed minimal clinical improvement despite some statistically significant changes. While reductions in DASS (1.38%), FAS (3.19%), PSS (2.59%), and SQI (1.97%) scores were statistically significant ($p < 0.05$), they were too minor to be considered clinically meaningful. These findings are consistent with the understanding that placebo effects often reflect psychological expectancy rather than therapeutic efficacy. Median values remained stable, and standard deviations exhibited negligible changes, suggesting no meaningful physiological or psychological shift.

Table 1 presents a comparative analysis between Group A (Standardized processed Ashwagandha root powder) and Group B (Placebo) at the end of the intervention period using the Mann–Whitney U test which was consistently low ($U = 37.00$), and p-values were uniformly 0.0001 across all variables, indicating highly significant intergroup differences.

Table 2 showed that the Group A exhibited a significant increase in hGH levels from 0.77 ng/mL at baseline to 1.09 ng/mL after treatment, reflecting a 40.78% improvement. The paired t-test yielded a t-value of 3.081 and a p-value of 0.003, confirming statistical significance. Although the standard deviation rose from 1.85 to 2.59, indicating greater variability, this may be attributed to individual biological responsiveness to standardized processed Ashwagandha root powder. These findings align with Ashwagandha's recognized adaptogenic potential in regulating endocrine responses and promoting physiological restoration. Group B demonstrated a non-significant decrease in hGH levels from 0.75 ng/mL to 0.55 ng/mL (26.38% decline), with a t-value of 0.716 and $p = 0.477$. The drop in standard deviation (1.79 to 0.71) suggests reduced variability, but the absence of meaningful change confirms the lack of biological impact in the placebo group. Graph 1 visually depicts the changes in hGH levels presented in Table 2, illustrating the significant increase observed in the Ashwagandha group compared with the minimal change in the placebo group.

Table 3 showed that the participants in Group A experienced a significant 40.78% rise in mean free testosterone levels, from 8.99 pg/mL to 12.58 pg/mL post-treatment. The t-value of 5.951 and $p < 0.0001$

Table 1 — Intergroup comparison of subjective parameters at Day 60

Variable	Group	N	Mean rank	Sum of ranks	Mann-whitney U	P-value	Result
DASS Score	Group A	52	75.50	3775.00	37.000	0.0001	HS
	Group B	52	25.50	1275.00			
	Total	100					
FAS Score	Group A	52	75.50	3775.00	37.000	0.0001	HS
	Group B	52	25.50	1275.00			
	Total	100					
PSS Score	Group A	52	75.50	3775.00	37.000	0.0001	HS
	Group B	52	25.50	1275.00			
	Total	100					
SQI Score	Group A	52	75.50	3775.00	37.000	0.0001	HS
	Group B	52	25.50	1275.00			
	Total	100					

DASS = Depression Anxiety Stress Scale; FAS = Fatigue Assessment Scale; PSS = Perceived Stress Scale; PSQI = Pittsburgh Sleep Quality Index, n = number of participants; Mann–Whitney U test used for between-group comparison; $p < 0.05$ considered statistically significant; HS = highly significant.

Table 2 — Effect on Human growth hormone (hGH) in both groups

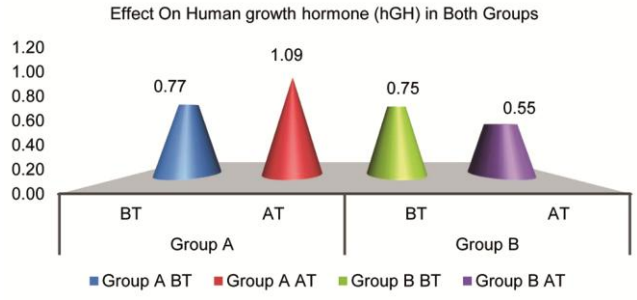
hGH		Mean	N	SD	SE	t-Value	P-value	% Change	Result
Group A	BT	0.77	52	1.85	0.26	-3.020	0.004	40.78	Sig
	AT	1.09	52	2.59	0.37				
Group B	BT	0.75	52	1.79	0.25	0.716	0.477	26.38	NS
	AT	0.55	52	0.71	0.10				

hGH = human growth hormone; , BT = Baseline; AT = After Treatment (Day 30); n = number of participants; SD = standard deviation; SE = standard error of mean; t-test used for comparison; $p < 0.05$ considered statistically significant; percentage change calculated from baseline to post-treatment.

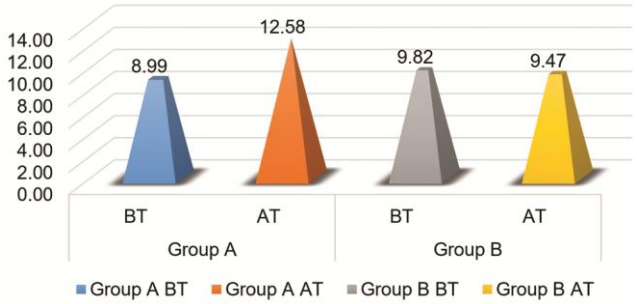
confirm a highly significant effect. While the standard deviation increased from 10.69 to 14.96, indicating variability in individual responses, the direction and strength of change reinforce Ashwagandha's positive influence on androgenic regulation[9,11]. Group B exhibited a non-significant 3.60% decrease, from 9.82 pg/mL to 9.47 pg/mL (t = 0.275, p = 0.784). Minor variation in standard deviation (11.72 to 12.12) suggests consistency without therapeutic gain. Graph 2 illustrates the changes in free testosterone levels presented in (Table 3), highlighting the significant increase observed in the Ashwagandha group compared with the negligible change in the placebo group.

Table 4 summarizes the intervention's effects using paired t-test comparisons. Group A showed a 40% increase in mean total testosterone, from 204.77 ng/dL at baseline to 286.67 ng/dL post-treatment. This rise was statistically significant (t = 6.425, p < 0.001). In the placebo group, total testosterone levels dropped by 20.68%, from 202.94 ng/dL to 160.97 ng/dL, but the change lacked statistical significance (t = 1.398, p = 0.168). This suggests normal biological fluctuation rather than a treatment-related effect. The consistent androgenic improvements in Group A, seen in both free and total testosterone, reinforce Ashwagandha's utility in managing stress-related hormonal dysregulation. Graph 3 illustrates the changes in total testosterone levels presented in (Table 4), clearly demonstrating the significant increase in the Ashwagandha group compared with the non-significant decline observed in the placebo group.

The intergroup comparison presented in (Table 5) highlights significant differences in key objective parameters—including human growth hormone (hGH), and testosterone levels—between the standardized processed Ashwagandha root powder-treated group (Group A) and the placebo group (Group B), assessed using the unpaired t-test. The analysis revealed statistically significant differences (p < 0.05) across most biomarkers, supporting the inference that the changes observed in Group A were not only internally consistent but also superior to those in the placebo group. These results affirm the therapeutic efficacy of Processed Ashwagandha Root Powder and diminish



Graph 1 — Effect on human growth hormone (hGH) in both groups



Graph 2 — Effect on testosterone free in both groups

Table 3 — Effect on testosterone free in both groups

Testosterone free		Mean	N	SD	SE	t-Value	P-value	% Change	Result
Group A	BT	8.99	52	10.69	1.51	-5.951	0.0001	40.03	Sig
	AT	12.58	52	14.96	2.12				
Group B	BT	9.82	52	11.72	1.66	0.275	0.784	3.60	NS
	AT	9.47	52	12.12	1.71				

BT = Baseline; AT = After Treatment (Day 30);); n = number of participants; SD = standard deviation; SE = standard error of mean; t-test used for comparison; p < 0.05 considered statistically significant; percentage change calculated from baseline to post-treatment, NS-Non Significant

Table 4 — Effect on testosterone total in both groups

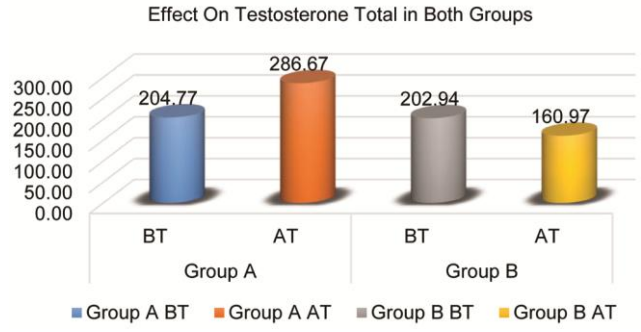
Testosterone total		Mean	N	SD	SE	t-Value	P-Value	% Change	Result
Group A	BT	204.77	52	225.36	31.87	-6.425	0.0001	40.00	Sig
	AT	286.67	52	315.50	44.62				
Group B	BT	202.94	52	227.79	32.21	1.398	0.168	20.68	NS
	AT	160.97	52	209.84	29.68				

BT = Baseline; AT = After Treatment (Day 30);); n = number of participants; SD = standard deviation; SE = standard error of mean; t-test used for comparison; p < 0.05 considered statistically significant; percentage change calculated from baseline to post-treatment, NS-Non Significant

the likelihood that the improvements were attributable to chance or placebo effects

Discussion

The present study evaluated the effect of standardized processed Ashwagandha root powder on stress, anxiety, exhaustion, sleep quality and several physiological indicators. Participants who received the intervention showed significant improvement in subjective outcomes, such as DASS, FAS, and PSS scores, as well as sleep quality as measured by PSQI. Similar observations have been observed in previous investigations pointing to the adaptogenic, anxiolytic and stress reducing characteristics of Ashwagandha[7-12]. Improvement in indices related to stress and sleep found may be due to regulation of the hypothalamic–pituitary–adrenal (HPA) axis and modulation of neurotransmitter activity, particularly GABAergic pathways[13,14]. The improvements were statistically significant, but should be interpreted with caution due to the length of the trial and the sample size. In the placebo group, most metrics changed only marginally. Few outcomes reached statistical significance but the magnitude of benefit was low and may not be clinically important. These kinds of responses are often shown in studies on stress-related disorders and may be due to placebo effects or normal oscillations in psychological state[15,16]. However, the intervention group had more robust and consistent gains across the variables tested, indicating a possible therapeutic effect of Ashwagandha beyond mere expectancy. But the prospect of sustained placebo effects is not out of the question. The comparative study between the two groups showed a significant difference in subjective assessment scores, revealing Ashwagandha to be more effective than placebo. The positive impacts were reported in several areas including reduction of stress, reduction of weariness, and improvement of the quality of sleep. These results validate the traditional use of Ashwagandha as a Rasayana and adaptogenic agent in Ayurveda[7,9,13]. However, given most measures were based on self-reported scales, the influence of reporting bias should also be taken into mind.



Graph 3 — Effect on testosterone total in both groups

Biochemical results showed a considerable increase in hGH levels in the intervention group, whereas no significant change was detected in the placebo group. This finding may suggest a potential favorable effect on endocrine function, however interpretation is limited due to the pulsatile nature of hGH secretion and absence of other stress related indicators such as cortisol[17,18]. Also, considerable rise in free and total testosterone levels was observed in Ashwagandha treated participants, consistent with past observations on improvement of androgenic activity with supplementation[9,11]. Also, when interpreting, hormonal variability and aspects like life style, food habits, baseline physiological status, etc. should be taken into consideration.

Overall, the results indicate a concurrent improvement of subjective symptoms and several hormonal indicators, hinting at a possible integrated action on stress physiology. However, explanations based on modification of the HPA axis or gonadal axis are speculative, as thorough endocrine profiling was not included of the present study[19-23]. The results of the study are clinically significant in that Ashwagandha may be beneficial for people with mild to moderate levels of stress, particularly in reducing felt stress, exhaustion and increasing sleep quality.

Table 5 — Intergroup comparison of objective parameters between Group A & Group B

Variable	Group	N	Mean Diff	SD	SE	t-Value	P-value	Result
Hgh	Group A	52	0.315	0.737	0.104	2.125	0.036	Sig
	Group B	52	-0.198	1.959	0.277			
Testosterone free	Group A	52	3.597	4.273	0.604	2.781	0.007	Sig
	Group B	52	-0.354	9.092	1.286			
Testosterone Total	Group A	52	81.904	90.139	12.748	3.797	0.000	Sig
	Group B	52	-41.979	212.358	30.032			

n = number of participants; SD = standard deviation; SE = standard error of mean; paired or independent t-test applied as appropriate; $p < 0.05$ considered statistically significant; interpretation based on exact p-values (significant if $p < 0.05$).

However, caution is warranted in making inferences about long-term safety, larger clinical applications, or definite endocrine consequences. Some drawbacks of this study include the moderate sample size, relatively short length, reliance on subjective questionnaires, and restricted hormonal analysis, which may limit the wider generalization of the findings.

In summary, the present findings are in line with the earlier published literature and suggest the potential function of standardized processed Ashwagandha root powder as an adjuvant method for stress management[7,9,13]. More well-designed studies with bigger sample sizes, longer follow-up periods and detailed neuroendocrine evaluation including cortisol and associated biomarkers are needed to confirm these findings and to elucidate the underlying mechanisms.

Study limitations

There are some drawbacks of this study. The moderate sample size and the short intervention and follow-up time may restrict the generalizability and inference of long-term effects. Reporting bias could be a limitation for subjective scales (PSS, DASS-21, PSQI). Hormonal indicators were measured, but comprehensive neuroendocrine markers (e.g., cortisol) were not analysed. No strict supervision on adherence and lifestyle factors (diet, activity, sleep habits) was applied. Additionally, the results are limited to people with mild to moderate stress and have not been tested in other populations.

Conclusion

The present double-blind, randomized, placebo-controlled clinical trial indicated the therapeutic effects of standardized processed Ashwagandha root powder in the management of stress and anxiety with improvement in sleep quality and several endocrine parameters. Standardized processed Ashwagandha root powder group showed significant improvements in subjective and objective measures like decrease in Perceived Stress Scale (PSS) and Depression Anxiety Stress Scale (DASS) scores, improvement in sleep quality as assessed by Pittsburgh Sleep Quality Index (PSQI) and increase in serum free testosterone, total testosterone and human growth hormone (hGH) levels. The placebo group demonstrated very minor alterations, most of which were not clinically meaningful. Overall improvements in the intervention group indicate that Ashwagandha's therapeutic effects are not limited to placebo response. The concomitant improvement of psychological symptoms and hormonal markers may be a signal of a more global adaptogenic effect on stress physiology, perhaps through the regulation of the hypothalamic-pituitary-adrenal (HPA) axis and related neuroendocrine systems. The findings of the present investigation support the potential usefulness of standardized processed Ashwagandha root powder as a natural and well-tolerated remedy in patients with stress-related symptoms, particularly when combined with disturbed sleep and changed hormonal status. However, the limited period of the trial and restricted hormonal profiling should be interpreted cautiously when considering endocrine results. Further research is needed to validate these findings and to further understand the long-term therapeutic and preventative effect of Ashwagandha in stress-associated physiological abnormalities with bigger sample numbers, longer follow-up periods and complete neuroendocrine examination.

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Author Contributions

PKP: Study design, supervision, conceptualization, and critical revision of text. HKS: Conception and design, data interpretation, drafting the article and final approval. GPG: Design of the study, clinical supervision, and critical assessment of the article. BS: Patient recruitment, clinical evaluation and data collecting. BC: Data gathering, patient follow-up and documentation. NS: Data collecting, coordination of clinical procedures and record keeping. DC: Statistical analysis, data interpretation and reviewing outcomes. AT: Study material, laboratory

coordination & technical support. MV: Manufacturing support, quality control of study product, and documentation. AG: Technical contribution, data management and editing of manuscript.

Conflict of Interest

The authors state that there is no conflict of interest with the present work. All authors had full access to all the data in the study and take complete responsibility for the integrity of the data and the accuracy of the data analysis.

Ethics Approval

The trial was approved by the Institutional Ethics Committee (IEC) of the Post Graduate Institute of Ayurved, Dr. Sarvepalli Radhakrishnan Rajasthan Ayurved University (DSRRAU), Jodhpur before beginning of trial.

Clinical Trial Registration Number

The trial was prospectively registered with Clinical Trials Registry–India (CTRI) with registration number CTRI/2024/09/074425.

Prior Informed Consent

Written informed consent was obtained from all individuals before inclusion in the study. All participants were given detailed information about the aim, procedures, potential advantages and voluntary nature of the involvement. To ensure confidentiality and privacy, participant information was anonymized at all times during the study.

Use of Artificial Intelligence (AI)

The authors used an AI-assisted language editing tool to improve the English language and readability of this manuscript. All intellectual content, data analysis, interpretations, and conclusions were developed and verified by the authors

Data Availability

The datasets created and analyzed during the current investigation are not publically available due to ethical and institutional restrictions on protecting participant confidentiality. However, de-identified participant data will be available from the corresponding author on reasonable request, as well as pertinent study materials (eg, study protocol, statistical analysis plan). All data sharing will be subject to the approval of the institutional ethics committee and to applicable ethical and regulatory constraints.

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