Stress-relieving effects of Ashwa 30 (*Withania somnifera* extract): A randomised, double-blind, placebo-controlled study

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Abstract

Background and Aim: Exposure to stressful events activates the hypothalamic–pituitary–adrenal (HPA) axis, leading to increased cortisol release and subsequent physiological and emotional alterations. The present study aimed to evaluate the effect of Ashwa 30 on cortisol levels released in response to stress.

Methods: A one-week, randomized, double-blind, placebo-controlled study was conducted in forty healthy male participants (n=20 per group). Participants received either Ashwa 30 (30 mg) or placebo once daily for a week. On day 7, the Maastricht Acute Stress Test (MAST)—a

validated and reliable non-invasive procedure for activating the stress system—was administered. Saliva samples were collected 10 minutes post-MAST, and stress-related changes were assessed by comparing post-MAST salivary cortisol levels between the two groups.

Results and Conclusion: Ashwa 30 supplementation significantly reduced salivary cortisol levels following MAST exposure compared with placebo (P = 0.03), indicating an optimized stress response. The intervention was well tolerated, with no reports of major adverse effects.

Keywords: Ashwagandha, *Withania somnifera*, Stress, Cortisol, Acute Stress

Introduction

Stress, lack of energy or fatigue are one of major factors that exacerbate the risk of life-threatening health issues. The lack of a holistic approach in conventional medicine to deal with stress has led to a shift towards the use of herbal products/ nutraceuticals, lifestyle changes and other therapies like cognitive - behavioural therapy among people. (1). "Adaptogens" are ideal solutions to these problems owing to their comprehensive health benefits.

Ashwagandha (*Withania somnifera*), a popular herb and a potent adaptogen in the Ayurvedic system of medicine has a well-documented history of use for millennia. (2) (3). Ashwagandha is classified as a 'Rasayana' (rejuvenator) which helps to promote physical as well as mental health and improve longevity. (4). Various Ayurvedic texts such as Caraka-Samhita (1000 BC), Susruta-Samhita (1000 BC), Kasyapa-Samhita (600AD), highlight the multifaceted use of the root of Ashwagandha in numerous human ailments. (5). *W. somnifera* is used alone or in combination with other plants for treating various diseases. Ashwagandha, a perennial shrub (family Solanaceae), is cultivated in dry and scorching parts of tropical and subtropical zones of world. (6).

Stress can be defined as state in which the body's internal balance or homeostasis is disturbed or disrupted. (7). Any stimuli (physical or psychological stressor) which disrupts homeostasis result in a stress response. A stress response is generally a combination of the physiological and behavioural alterations that manifest while encountering a stressor. The biological response to stress is mediated through a complex network of multisystem mechanisms which lead to the activation of the Sympathetic-Adreno-Medullar System and Hypothalamic-Pituitary-Adrenal System, the 2 major pillars of the stress response (8). The sympathetic-adreno-medullar (SAM) axis, secretes noradrenaline and norepinephrine and the hypothalamus-pituitary-adrenal (HPA) axis, secretes glucocorticoids including cortisol as a stress response. (9).

Acute stress has significant implications across diverse occupational settings. In professions exposure to unpredictable and threatening situations demands rapid decision-making under intense physiological and psychological arousal. This acute stress response, mediated through activation of the autonomic nervous system and the hypothalamic–pituitary–adrenal (HPA) axis, can impair cognitive flexibility, working memory, and fine motor skills, thereby affecting judgment, reaction time, and task execution. Beyond tactical occupations, acute stress is also prevalent in healthcare, education, and corporate sectors, where high workload, time pressure, and performance expectations can precipitate errors, reduce efficiency, and increase emotional exhaustion. Collectively, these effects underscore the importance of developing interventions to optimize stress regulation and preserve performance in both high-stakes and everyday work environments (10).

The current spike in the demand for ashwagandha has led to the emergence of root or root & leaf based ashwagandha products. This unmet demand calls for a standardised, root-only, low dose ashwagandha product with established efficacy and safety. The present study was designed to evaluate the effect of Ashwa 30, a unique low-dose Ashwagandha extract developed through Bioactive Optimization Technology, on cortisol release under acute stress conditions in healthy participants following seven days of supplementation.

Methods

Study design

The two-arm randomised, prospective, double-blind, placebo - controlled clinical study was conducted at Natural Remedies Private Limited, Bengaluru, India.

Recruitment and randomisation

After signing the informed consent, eligible participants were randomly allocated to Ashwa 30 or placebo group in 1:1 ratio through a simple randomisation method. To ensure sequence concealment, the randomization sequence was completed by a researcher not involved in participant recruitment and details of the bottle codes held with sponsor were revealed after data collection.

Participants

Inclusion criteria

Healthy male adults aged 25 -55 years were recruited after a routine health checkup. Participants who were willing to provide a personally signed and dated informed consent form with details of the study and willing to abstain from all dietary supplements were recruited.

Exclusion criteria

Participants suffering from or have a history of medical conditions such as but not limited to diabetes mellitus, hypertension, other cardiovascular diseases, gastrointestinal disorders, endocrine disorders, infectious diseases, acute or chronic pain conditions, neurological disorders, psychiatric disorders and skin conditions that prevent them from taking the test.

Participants were deemed ineligible if they were taking vitamin or mineral supplements, anxiolytics, antidepressants, or any treatment likely to affect salivary cortisol levels, including topical steroids. Individuals with a history of illicit drug use, major surgical interventions, current use of medications or food supplements for any disease or disorder or known hypersensitivity to the investigational product were excluded. In addition, participants who had experienced a significant life event within two weeks prior to enrollment or anticipated such an event during the study (e.g., bereavement, divorce, major changes in professional activities, or travel associated with jet lag) were not eligible. Shift workers, individuals working in extreme environmental conditions (e.g., cold rooms), those engaged in high levels of physical activity, or those with habitual consumption of more than three cups of coffee in the morning or more than three alcoholic drinks per day were excluded. Further restrictions required that participants maintain consistent dietary, sleep, and exercise routines throughout the study period.

Intervention

Withania somnifera also known as Ashwagandha (Family: Solanaceae) roots were sourced from southern parts of India. The authentication was done, and voucher specimen number 128101 is kept in the Herbarium of the Foundation for Revitalisation of Local Health Traditions (FRLHT) in Bengaluru.

The test substance used was a proprietary extract (Pending patent: 202541047628) derived from the roots of *Withania somnifera*. It was extracted with ethanol and water to achieve a standardization of withanolides (>15% w/w determined using a modified USP HPLC method). In addition to it, the extract was also standardized to ATP active fraction (>15% w/w).

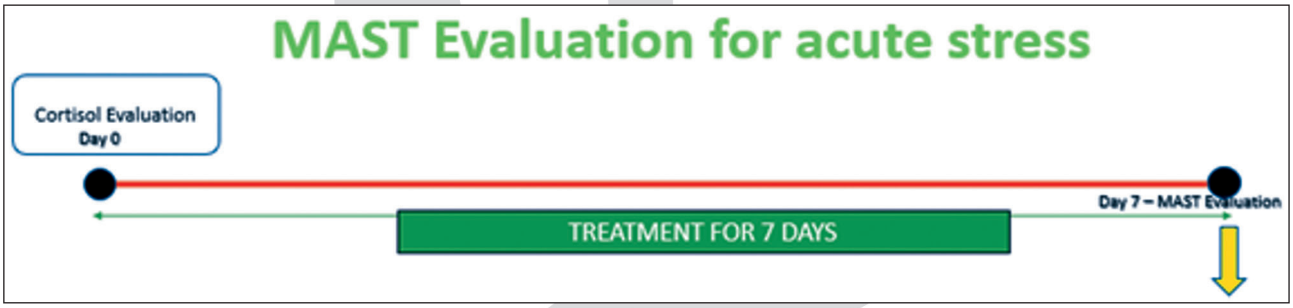
Ashwa 30 capsules containing 30 mg of standardised root- only Ashwagandha extract (Ashwa-30™) were used as the test product. Capsules containing microcrystalline cellulose and syloid 244 FP were used as the placebo. Both Ashwa 30 and placebo capsules were manufactured in a Good Manufacturing Practice (GMP)-certified facility. The test products complied with the United States Pharmacopeia (USP) requirements for dietary supplements, including limits for microbial contaminants, heavy metals, residual pesticides, aflatoxins and residual solvents. Each bottle dispensed to subjects contained capsules of either Ashwa 30 or placebo and was labelled with a unique randomization number. Subjects were instructed to take one capsule daily in the morning, either before or after food, for 7 days *(Figure 1)*. The Ashwa 30 and placebo capsules prepared in such a way to make sure that they were identical in appearance and were packaged in a manner to ensure blinding.

Outcome measure

Maastricht acute stress test - MAST

MAST is a simple, swift and non-invasive valid and reliable procedure designed to stimulate a stress response (11) (12).

MAST begins with a 5min preparation/ room acclimatization phase. The participants are briefed about the upcoming task and the instructions are given. During the 12min MAST *(Figure2),* participants were required to place their hand in cold water at 7◦C and complete an arithmetic task (counting backward by 7s) for alternating time intervals. Five hand immersion trials were alternated with four mental arithmetic tasks. 5 ml of saliva was collected at Day 0, Day 7 before MAST and Day 7 after MAST for cortisol evaluation (from 2.30 pm to 5 pm due to the diurnal cycle of cortisol). On the day of analysis, samples were thawed and precipitated mucins and particulate debris were separated by centrifugation at 1,500 g for 25min at room temperature. An aliquot of the supernatant was used for the measurement of cortisol. Salivary cortisol was measured using Electrochemiluminescence Immunoassay (ECLIA).



CPT- Cold Pressor Test, MAT- Mental Arithmetic Test

Figure 1. Trial protocol

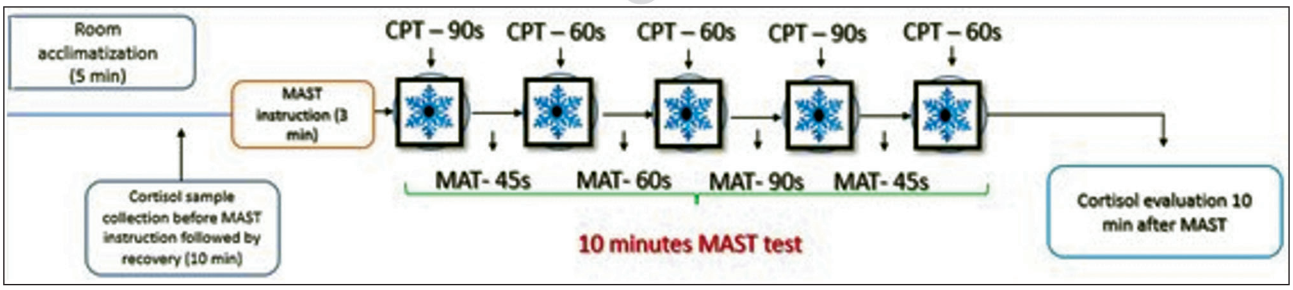


Figure 2. MAST test Procedure

Salivary cortisol levels before and after acute stress induction was measured as outcome of the study. Salivary measure was chosen as it is non-invasive, inducing minimal or no stress and also is a validated method to measure HP axis in stress response (13)

Safety assessments

Safety assessments included adverse events monitoring, vital signs and general physical examination. AEs were monitored continuously throughout the study. Vital signs (SpO2, blood pressure, respiratory rate, pulse rate, and body temperature) and physical examinations were performed at each study visit.

Statistical analysis

Quantitative variables were reported as mean ± standard deviation. Within-group comparisons were performed using a paired t-test or Wilcoxon signed-rank test, depending on data distribution. Between-group comparisons were conducted using an unpaired t-test or Mann-Whitney U test. Normality tests (Kolmogorov-Smirnov or Shapiro-Wilk) were conducted to assess data distribution assumptions. All statistical tests were two-sided, with a significance threshold set at $p<0.05$.

Results

Out of 44 adult men who were willing to participate in the study, 40 participants were eligible based on the inclusion and exclusion criteria. Participants were assigned either to Ashwa 30 or placebo group.

Salivary cortisol levels

Table. 1 depicts the changes in the cortisol levels at Day 7 before and after MAST in both Ashwa 30 and placebo groups. There was no significant difference with respect to salivary cortisol levels of Ashwa 30 and placebo pre-MAST. The rise is salivary cortisol post MAST was significantly less compared to placebo. Although there is a significant increase in the salivary cortisol levels in the Ashwa 30 group after MAST compared to the before MAST levels, the increase is highly significant in the Placebo group.

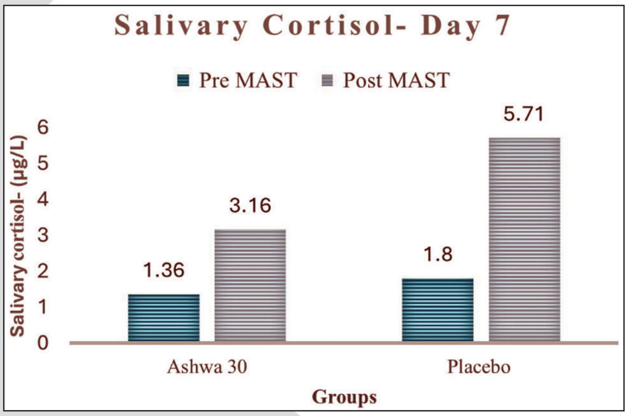


Figure 3. Effect of Ashwa 30 on salivary cortisol

Safety assessments

No adverse events were reported during the study. No abnormalities were observed in the vital signs and physical examinations.

Discussion

Acute stress is known to significantly impair performance and decision-making, reduce efficiency, and induce exhaustion; moreover, repeated exposure over the long term can have profound adverse consequences on overall health and functioning.

This study aimed to analyse the efficacy and safety of Ashwa 30 in managing acute stress. The study results revealed that Ashwa 30 extract signi-

Table 1. Salivary cortisol levels- pre and post MAST

Salivary cortisol (µg/L) - Day 7			
	Pre MAST	**Post MAST**	**p value** *(within group)*
Ashwa 30	1.36 ± 0.96	3.16 ± 2.02	0.02*
Placebo	1.80 ± 0.73	5.71 ± 3.83	0.0008*
p value *(Ashwa 30 vs Placebo)*	0.1856	0.0387#	

** P < 0.05 = statistically significant in within group, otherwise the difference was not statistically significant.*

 # P < 0.05 = statistically significant in between groups, otherwise the difference was not statistically significant.

Data were analyzed using an unpaired t-test for between-group comparisons and a paired t-test for within-group comparisons.

ficantly reduced the salivary cortisol levels post-acute stress induction. While there are multiple studies on Ashwagandha from root /root and leaf extracts studied the effects of intervention on cortisol in chronic stress (14). This is the first study that investigated the effect of Ashwa 30 on cortisol levels in acute settings.

It is noteworthy to highlight that the cortisol awakening response was not altered by Ashwa 30 after acute stress induction, but the increase was significantly lesser compared to placebo. This is beneficial because stress response is essential for survival. Thus, Ashwa 30 aids in optimizing stress regulation and preserves performance in work environment & other high-stake environments.

Ashwa 30 possibly reduces stress by modulating the hypothalamic-pituitary-adrenal (HPA) axis and thereby reducing the cortisol levels. The well-established relationship between stress and neuropsychiatric disorders, highlights the speciality of ashwagandha's anti-stress activity and its potential health benefits. (15).

A key strength of this study is its focus on examining the activity of Ashwa 30 in stress management after a short, 7-day consumption period.

While Ashwa 30 demonstrated significant efficacy in managing acute stress after 7 days, further studies with a larger subject pool and longer durations are needed to fully substantiate its efficacy and safety.

Conclusion

In conclusion, standardised root-only, Ashwa 30 ™ proves its efficacy in managing acute stress by significantly reducing salivary cortisol levels. No serious adverse events were observed during the study.

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