An Evaluation of Efficacy and Safety of Ashwadaat® botanical extract in patients with Stress and Insomnia – An Open label, multi center, non-comparative, interventional, prospective clinical study

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Abstract:

***Introduction**: Ashwadaat® is a proprietary extract based on standardized Ashwagandha (Withania somnifera) extract, which is a herb possessing anti-stress, anti-anxiety, anti- depressant, and sleep inducing properties.*

***Objective**: The objective of the study was to evaluate efficacy and safety of Ashwadaat® in individuals with Stress and Insomnia.*

***Methods**: In an Open label, multi centre, non-comparative, interventional, prospective clinical study carried out at two study sites, 30 individuals suffering from Stress and Insomnia were considered for the study after obtaining ethical clearance & informed consent. Study participants were advised to take Ashwadaat® in a dose of 2 Capsules twice daily (500 mg Ashwadaat®) for a period of 30 days. Assessment of efficacy was done by stress by Perceived Stress Scale (PSS), severity of Insomnia on Insomnia Severity Index, assessment of quality of life (QOL) on GHQ28 scale and Serum Cortisol level (Morning). Safety related blood investigations were done for assessment of safety.*

***Results**:*

There was statistically significant reduction in stress and insomnia with the use of Ashwadaat®. A significant improvement in sleep quality along with improvement of quality of life (QOL) was seen at day 15 and day 30 as compared to the baseline scores. There was a reduction in morning Serum cortisol levels (within normal range) from baseline to 30 days. Excellent tolerability was reported over 30 days of consumption of Ashwadaat®. There were no adverse effects due to the consumption of Ashwadaat® and also the safety related laboratory parameters were within the normal range at baseline and further at the end of the study.

***Conclusion**: Ashwadaat® was found to be effective in reduction of stress and other associated symptoms, also effective in improving sleep quality, quality of life, without producing any adverse effects.*

Introduction:

Stress is said to be the body's method of reacting to a challenge. It is known as the physical, emotional or intellectual strain caused as a result of our response to environmental stressors. In humans, stress typically manifests as a negative or positive condition that impacts a person's mental and physical wellbeing.[1] Excess stress often manifests itself in a variety of emotional, behavioral, and physical symptoms. Symptoms of stress vary enormously between different individuals. Common somatic symptoms often reported by those experiencing excess stress include sleep disturbances or changes in sleeping habits (insomnia, excess sleeping), muscle tension, muscle aches, headache, gastrointestinal problems, and fatigue.[2]

Insomnia is a condition also known as sleeplessness. Individuals experiencing insomnia may have difficulty either falling asleep or staying asleep as long as desired.[3,4] Insomnia can occur independently or as a result of secondary effects of other conditions such as psychological stress, chronic pain, hyperthyroidism, heartburn, restless leg syndrome, menopause, certain medications, and drugs such as caffeine, nicotine and alcohol.[5,6,7] Other risk factors include working night shifts and sleep apnea.[4] Immediate effects of insomnia include low energy, daytime sleepiness, irritability and a depressed mood.[3] Females and elderly people are more prone to insomnia.[6]

Lifestyle changes and sleep hygiene are typically the first line treatment for stress and insomnia.[8] Other lifestyle changes include consistent exercise and cognitive behavioral therapy (CBT), Meditation and yoga. [9] Tranquillizers and antidepressants can help to reduce or manage some of the signs of stress and insomnia. But these medications include side effects which may cause nausea, increased appetite, weight gain, fatigue, drowsiness, dry mouth, blurred vision and constipation in a significant proportion of users.[10]

Many single herbs and poly-herbal formulations have been studied for the treatment of stress and insomnia. Ashwadaat® is a proprietary extract developed by Lodaat LLC and is based on Ashwagandha (*Withania somnifera)* extract; a herb which possesses anti-stress, anti-anxiety, anti- depressant, and sleep inducing

properties.[11,12] *Ashwagandha* has been effectively used for the management of stress and insomnia.[13] In this trial, Ashwadaat ® (*Withania somnifera*) Extract was blended with *Piper Nigrum* Extract (for bioavailability enhancing property).[14,15]

Based upon the constituents of Ashwadaat®, a hypothesis was postulated that it would be useful in the management of stress and insomnia. To test the hypothesis, the present clinical study was planned. The objective of the study was to evaluate efficacy and safety of Ashwadaat® in patients with Stress and Insomnia.

Materials & Methods-

- **Study design, sites –**
  This was an open label, multi centre, non-comparative, interventional, prospective clinical study carried out at two study sites in Maharashtra viz. D. Y. Patil University School of Ayurveda and Hospital, Nerul, Navi Mumbai and KVTR Ayurvedic College, Boradi, Dhule.
- **Ethical considerations-**
  Ethical approvals from Institutional ethics committees of all study centers were taken and it was registered on Clinical Trials Registry of India (CTRI) website vide registration number, CTRI/2020/11/028937 dated 15th November 2020.
- **Enrolment of patients-**
  Patients, suffering from Stress and Insomnia attending out-patient department of the study centers and who consented, were considered for the study. The study was carried out and reported adhering to CONSORT statement.
- **Study duration & Visits-**
  The total duration of the study treatment was 30 days. Patients were asked to visit study site every 15th day for 1 month. Patients were evaluated for efficacy and safety assessment by clinical examination and various scales.
- **Inclusion Criteria-**
  Patients of either sex in the age group of 18 to 55 years (both inclusive) who perceived themselves to be under stress and were having a score between 14 -24 (mild to moderate) on the Perceived Stress Scale (PSS), not having any psychiatric conditions other than stress and who were willing to follow the procedures as per the study protocol and voluntarily sign an informed consent form were included in the study.

  Exclusion Criteria:

  Patients suffering from any chronic physical, hormonal, or psychiatric illness, on treatment for stress and insomnia, using oral or systemic contraceptive medications, suffering from uncontrolled diabetes and/or hypertension, with substance dependence, chronic alcoholics and habitual tobacco chewers were excluded from the study. Individuals with known cases of Severe/Chronic hepatic or renal disease, any active malignancy, history of significant cardiovascular event < 12 weeks prior to recruitment, with known chronic, contagious infectious disease, such as active tuberculosis, Hepatitis B or C, HIV, and metabolic or gastrointestinal diseases that could have interfered with nutrient absorption, metabolism, or excretion, excluding diabetes were excluded.

  Patients on any other investigational drug within 1 month prior to recruitment or patients were participating in any other clinical study, known hypersensitivity to any of the ingredients used in study drug, Pregnant and Lactating females and any other condition due to which patients were deemed to be unsuitable by the investigator for reason(s) not specifically started in the exclusion criteria were excluded from the study.
- **Laboratory Investigations:**
  Blood investigations i.e. CBC, Hb%, blood sugar fasting, liver function tests, renal function tests, lipid profile, morning serum cortisol level and HIV test (if necessary) were performed. Also, patient's ECG and X-ray chest (if necessary) were done.
- **Details of Study Intervention (Study Drug) –**
  The study drug was Ashwadaat® a proprietary ingredient extract based on Ashwagandha (*Withania somnifera*). Patients were advised to take Ashwadaat® in a dose of 2 Capsules twice daily (500mg Ashwadaat®) for a period of 30 days.
- **Sample size:**
  A total of 32 patients were screened in the study, out of which there were 2 screen failures who did not meet the inclusion criteria. A total of 30 patients were recruited in the study. The details are presented in the CONSORT provided in the annexure.

  Assessment of Efficacy Parameters:

  Assessment of stress on Perceived Stress Scale (PSS)[16] and severity of Insomnia on Insomnia Severity Index[17] were performed at screening visit, Baseline Visit, Visit 1 (Day 15) and Visit 2 (Day 30). Serum Cortisol level (Morning) was checked on screening visit and Visit 2 i.e. End of study Visit (Day 30). Assessment of quality of life (QOL) on GHQ28 scale[18] was done on baseline visit, visit 1and visit 2. Assessment of changes in Vitals: Assessment of changes in vitals including Pulse rate, Respiratory rate, Temperature and Blood pressure were measured at every follow up visit and were compared to each participant.

Assessment of overall efficacy on CGI-I: CGI-I was recorded/filled at the end of the study. The full CGI-I scale assessment measures are indicated below.

Table 1: Assessment of overall efficacy on CGI-I

0 = Not assessed	4 = No change
1 = Very much improved	5 = Minimally worse
2 = Much improved	6 = Much worse
3 = Minimally improved	7= Very much worse

Assessment of Subject's Global evaluation for overall change: The subject rated the total change, whether or not, it was entirely due to product treatment compared to his / her condition at admission to the study and how much has he/she changed.

Table 2: The tolerability of Ashwadaat® was evaluated on following safety grades as;

1	excellent overall safety (no adverse event/s reported)
2	good overall safety (mild adverse events (s) reported which subside with or without medication)
3	fair overall safety (moderate to severe adverse event(s) reported which subside with or without medication and do not necessitate stoppage of study treatment)
4	poor overall safety (severe or serious adverse event(s) which necessitate stoppage of study

Assessment of Safety:

Safety was assessed by clinical review of all safety parameters, including the adverse event reporting, as applicable, Vital signs including allergic reactions etc., Laboratory parameters like Liver function tests (LFT), Renal function tests (RFT), complete blood count (CBC), ESR, Hb%, assessment of Overall Safety and Tolerability of the study drug by the physician and subject on global assessment scale by the investigator and by subject himself**.**

All Adverse Events data were listed per subject including severity grading, relationship with investigational product and relationship of the adverse event to other causality, action taken and outcome of the adverse event. Any clinically significant changes in laboratory parameters were reported.

- **Statistical methods-**

All the subjects who took at least one dose of the study drug and who gave at least one post baseline follow up were considered for safety evaluation in the study. All the subjects who completed the study as per the protocol were considered as "Per Protocol Population". Also, all cases who took at least one dose of the study drug were considered as "Safety population" and were evaluated accordingly.

The study data generated and collected was put to statistical analysis to reach to the final results and conclusions. The demographic data are presented in tables and graphs. The data on discrete variables has been represented as actual numbers and percentage. The data on continuous variables has been represented as Mean ± SD. GraphPad InStat Version 3.6 (www.graphpad.com) software was used for statistical analysis of data. $p < 0.05$ was considered significant. For continuous data – Intra-group comparison, Paired t test, Wilcoxon matched- pairs signed-ranks test was used. For discrete data (counted facts), non - parametric test, i. e. Chi – Square test was applied.

Observations & Results-

A total of 32 subjects were screened in the study, out of which there were 2 screen failures who did not meet the inclusion criteria. A total of 30 subjects were recruited in the study. All 30 subjects were considered to be completers or efficacy evaluable cases at the end of the study. There were no dropouts in the study.

Demographic details - There was a total of 21 (70%) male patients and 9 (30%) female patients in the study. The average age of 30 patients in the study was 41.47 ± 8.04. Maximum number of patients (43.33%) were in the age group of 25 to 35 years followed by (26.66%) between the age group of 46-55 years.

Clinical Assessment of Primary outcomes:

- Assessment of stress on perceived stress scale (PSS): It was observed that the mean PSS score at baseline was 23.81 ±4.59 which reduced to 18.76 ±1.51 at the end of 15 days and further to 13.71 ±3.95 at the end of 30 days of the study. There was a statistically significant reduction ($p<0.05$) in stress as measured by PSS at day 15 and day 30.

Table 3: Changes in severity of Insomnia on Insomnia Severity Index (Individual questions)

PSS Score	Baseline (Day 0)	Day 15	Day 30
Mean ± SD	23.81 ± 4.59	18.76 ± 1.51	13.71 ± 3.95

*$p < 0.05$ as compared to baseline values

Clinical Assessment of Secondary outcomes:

- **Severity of Insomnia on Insomnia Severity Index:** It was observed that the mean score of Insomnia Severity Index was 18.43 ±7.17 which reduced to 13.90 ±4.82 at the end of 15 days and further to 8.29 ±2.13 at the end of 30 days of the study. There was a statistically significant reduction ($p<0.05$) in the insomnia severity index score as measured on the scale at day 15 and day 30.

Table 4: Changes in severity of Insomnia on Insomnia Severity Index

Insomnia Severity Index Score	Baseline (Day 0)	Day 15	Day 30
Mean ± SD	18.43 ± 7.17	13.90 ± 4.82*	8.29 ± 2.13*

*$p < 0.05$ as compared to baseline values

Table 5: Changes in severity of Insomnia on Insomnia Severity Index (Individual questions)

Insomnia Index Question	Baseline (Day 0)	Day 15	Day 30
Difficulty falling asleep	2.62 ±0.49	2.05 ±0.22*	1.14 ±0.36*
Difficulty staying asleep	2.33 ±0.58	1.57 ±0.59*	1.36 ± 0.53*
Problem waking up too early	2.29 ±0.46	1.67 ±0.48*	1.19 ±0.40*
Dissatisfaction with current sleep pattern	2.86 ±0.36	2.05 ±0.22*	1.19 ±0.40*
Impairment in Quality of life	2.71 ±0.46	2.14 ±0.36*	1.24 ±0.44*
Worried/distressed about sleep problem	2.76 ±0.44	2.14 ±0.36*	1.24 ±0.44*
Interference in daily life	2.86 ±0.48	2.29 ±0.56*	1.29 ±0.46*

*$p < 0.05$ as compared to baseline values

- **Change in pre and post serum cortisol (morning) level**: Serum cortisol levels (Morning) were checked at the baseline visit (using blood samples) and subsequently at the end of the study i.e. 30 days. The mean baseline cortisol level was 9.60 ±3.24 which showed a reduction at the end of the study to 8.73 ±2.65. The cortisol levels were found to be within the normal range at both baseline and at the end of the study.
- **Quality of Life on GHQ28 Scale**: GHQ28 score was used to analyze the quality of life of patients using Ashwadaat® for a period of 30 days. It was observed that the mean GHQ28 score at the study baseline visit was 42.48 ±7.17 which significantly reduced to 29.24 ± 3.43 at the end of 15 days and further to 20.05 ±3.43 at the end of 30 days. These improvement in the scores were found to be significant as compared to the baseline scores.
- **Global assessment of overall change as per the investigator and the subject:** The table below provides details of global assessment of overall efficacy as evaluated by the investigator. It was observed that a majority of patients showed very much improvement (73.33%) while 26.66% showed much improvement in their stress condition. None of the patients showed worsening in their overall condition. Similarly, overall assessment of change as per the subject's self-assessment showed that 70% of the patients reported of very much improvement, 26.66% of patients reported of much improvement and 3.33% of minimal improvement.
- **Global assessment of overall safety as per Investigator & subject**: Global assessment of overall safety as per the Investigator and the patients showed that all the patients showed excellent to good overall safety. None of the patients showed poor tolerability to Ashwadaat®.
- **Assessment of effect of study drug on Lab Parameters:** Laboratory parameters were done to evaluate the safety of consumption of Ashwadaat® for a period of 30 days. It was observed that there was no significant difference of the safety related laboratory parameters and the levels were within the normal range at baseline and further atthe end of the study.

Discussion:

Excessive stress is detrimental on many levels to humans, and it activates the defense system of the central nervous system. Stress-related physiological responses differ depending on each individual cognitive form, and

these physiological responses cause the neuro-endocrine responses and behavioral responses. Sleep is an essential biological process for humans. Many anatomical structures and biochemical substances are involved in the regulating mechanisms including the HPA axis, which is activated by the factors including stress and immune function. The regulation of sleep is configured with the circadian process that determines the beginning and ending of sleep, and the homeostatic process that maintains the depth and the amount of sleep.[19]

The present study was conducted to evaluate efficacy and safety of Ashwadaat® in subjects with stress and insomnia. Subjects were advised to take Ashwadaat® in a dose of 2 Capsules (500mg Ashwadaat®) twice daily orally for a period of 30 days.

A total of 32 subjects were screened in the study, out of which there were 2 screen failures as these subjects did not meet the inclusion criteria. A total of 30 subjects were recruited in the study and all these subjects were considered as completers or efficacy evaluable cases at the end of the study. There were no drop outs in the study. The average age of subjects in the study was 41.47 ±8.04 years. There was a total of 21 (70%) male subjects and 9 (30%) female subjects in the study.

In the present study, stress level was evaluated using perceived stress scale (PSS). The mean PSS score at baseline was 23.81 ±4.59 which significantly ($p<0.05$) reduced to 13.71 ±3.95 at the end of the study (day 30). Distress among subjects was evaluated using general health questionnaire which consists of 28 questions (GHQ28). It was observed that the mean GHQ28 score at baseline visit was 42.48 ±7.17 which significantly ($p<0.05$) reduced to 20.05 ±3.43 at the end of 30 days. As per GHQ28, a total score of 23/24 is the threshold for the presence of distress. It was observed that at the end of the study the mean total GHQ28 score significantly reduced to well below 23 suggesting no distress among the study subjects. Ashwagandha extract has been reported to possess stress relieving effects in earlier studies as well.[14] Recent studies show that Ashwagandha possesses adaptogens which could induce a state of non-specific increase of resistance to affect the internal homeostasis. The adaptogens improve the response to stress and help the body to adapt by normalizing physiological processes in times of increased stress.[20]

It was observed that the mean score of Insomnia Severity Index was 18.43 ±7.17 which reduced significantly ($p<0.05$) to 8.29 ±2.13 at the end of the study. The insomnia severity indexed was further analyzed for individual questions and it was observed that there was a significant reduction in the various symptoms associated with insomnia which included difficulty in falling asleep, difficulty in staying asleep, problems waking up too early, dissatisfaction with current sleep pattern, impairment of quality of life, worry/distress about sleep problem, interference with daily life. The sleep-inducing capacity of Ashwagandha has already been established in the clinical studies.[15] Ashwagandha possesses GABA-like activity which may account for its anti-anxiety effect. Due to GABA-like activity, Ashwagandha decreases neuronal activity, inhibits nerve cells from over firing and as a result, uplifts mood. This produces calming effect and can lead to stress reduction and induction of high quality sleep.

The baseline serum cortisol was 9.60 ±3.24 which showed a reduction to 8.73 ±2.65 at the end of the study. The cortisol levels were found to be within the normal range at both baseline and at the end of the study. As per global assessment of overall efficacy as evaluated by the investigator and subjects, a majority of subjects showed very much improvement in their stress and insomnia conditions. None of the subjects showed worsening in their overall condition. These findings suggest that Ashwadaat® effectively reduces stress and induces good quality sleep in subjects suffering from stress and insomnia.

Global assessment of overall safety as per the Investigator and the subjects showed that all the subjects showed excellent to good overall safety. None of the subjects showed poor tolerability to Ashwadaat®. There was a total of 8 adverse events recorded in the study. These adverse events were hyperacidity, abdominal discomfort, and constipation. None of the adverse events were reported to be due to the consumption of Ashwadaat®. There was no SAE reported in the study. Vital parameters were within normal range at baseline and further at follow up visits. It was observed that there was no significant difference of the safety related laboratory parameters and the levels were within the normal range at baseline and further at the end of the study. These findings confirm safety of Ashwadaat®.

Conclusion:

The present study concludes that Ashwadaat® helps in reduction of stress and other associated symptom, also effective in improving sleep quality. The product was also found to be safe without producing any adverse effects. Further comparative studies with larger sample size are warranted to validate and confirm on the clinical efficacy as well as mode of action of Ashwadaat®.

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