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Clinical evaluation of the pharmacological impact of ashwagandha root extract on sleep in healthy volunteers and insomnia patients: A double-blind, randomized, parallel-group, placebo-controlled study

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ABSTRACT

Ethnopharmacological relevance: Ashwagandha (*Withania somnifera* (L.) Dunal.) is long known for its sleep-inducing effects. Ashwagandha can be proposed as an alternative to the recommended present treatments for insomnia. This study aimed to evaluate the pharmacological effect of Ashwagandha root extract on sleep in healthy subjects and also in the subjects having insomnia.

Material and methods: We performed a randomized, parallel-group, stratified design, placebo-controlled study. A total of 80 eligible participants, 40 in Arm-A (healthy) and 40 in Arm-B (insomnia) were assigned to two groups, either Ashwagandha or placebo and studied for 8-weeks. The assessment was done based on the sleep parameters (Sleep Onset Latency, Total Sleep Time, Wake After Sleep Onset, Total time in bed, and Sleep Efficiency), Pittsburgh Sleep Quality Index and Hamilton Anxiety scale-A questionnaire, mental alertness on rising assessment, and sleep quality questionnaire. Safety and adverse events along with the concomitant medication were also assessed.

Results: In both healthy and insomnia subjects, there was a significant improvement in the sleep parameters in the Ashwagandha root extract supplemented group. The improvement was found more significant in insomnia subjects than healthy subjects. Repeat measure Analysis of variance (ANOVA) confirmed the significant improvement in SOL (p 0.013), HAM-A outcomes ($p < 0.05$), mental alertness (p 0.01), and sleep quality ($p < 0.05$) of the insomnia patients. A two-way ANOVA was used to confirm the outcomes that denoted sleep onset latency ($p < 0.0001$) and sleep efficiency ($p < 0.0001$) as the most improved parameters, followed by TST ($p < 0.002$) and WASO($p < 0.040$). All these parameters (SOL, TST, WASO, TIB, SE, PSQI, HAM-A, Mental Alertness, and Sleep quality) were also statistically assessed for the significant improvement within the group both for the treatment, and the placebo groups in the healthy and the insomnia datasets. Obtained results suggest statistically significant ($p < 0.0001$) changes between the baseline values and the end of the study results except for the HAM-A and the mental alertness scoresn the healthy subject group.

Conclusion: The present study confirms that Ashwagandha root extract can improve sleep quality and can help in managing insomnia. Ashwagandha root extract was well tolerated by all the participants irrespective of their health condition and age. Additional clinical trials are required to generalize the outcome.

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1. Introduction

Sleep is one of the most important physiological aspects of human health that helps in recycling energy, vitality, and rejuvenation. Insomnia has become the most common sleep disorder with a high prevalence rate (Morin et al., 2006). The condition is characterized by troubled sleep quality or insufficient sleep duration, physical or mental difficulty falling asleep, intermittent or incomplete sleep, and non-restorative or poor sleep quality (Morin and Benca, 2012). Due to the impaired quality of life and cognitive functions, insomnia is regarded as a clinical condition (Ranjbar et al., 2018). The clinical condition can be situational, recurrent or can turn into a persistent problem over time (Morin and Benca, 2012). Therefore, persistent insomnia can get associated with depression and anxiety disorders (Baglioni et al., 2011; Fernandez-Mendoza and Vgontzas, 2013). Such health conditions inevitably increase healthcare costs along with a gradual deterioration of the quality of life (Leger and Bayon, 2010).

Chronic primary insomnia occurs due to the sleep-initiation or sleep-maintenance problem for at least a month, while secondary insomnia induces due to medical, psychological issues, and drug reaction (Vgontzas et al., 1998, 2011). As insomnia is both a symptom and a disorder in itself, detailed self-evaluation of the problem is imperative before the clinical diagnosis (Sahoo, 2010). Even though polysomnography (PSG) is the accepted gold standard for sleep assessment, there is considerable variability in the sleep pattern of the insomniacs. Moreover, a minute evaluation may produce valuable results that may not be obtained from an individual's usual sleep pattern. Often, clinical and psychological conditions evaluation is mandatory for precise diagnosis. The application of Actigraphy enabled us to evaluate and monitor sleep patterns with a detailed data recording. Actigraphy helps in recording and integrating precise movements of the limbs of a person over time.

The use of device-based monitoring, such as actigraphy, is accurate, continuous, and effortless. Actigraphic devices are put on the wrist or ankle for sleep monitoring (Smith et al., 2018; Ancoli-Israel et al., 2003).

Pharmacotherapy and psychotherapy are the conventional treatments for insomnia (Morgenthaler et al., 2006; Schutte-Rodin et al., 2008). Effective pharmacological treatments include short and long-acting benzodiazepines. However, many such drugs cause adverse effects and daytime sedation, and patients become dependent on continued use (Gillin and Byerley, 1990). Cognitive-behavioral therapy for Insomnia (CBT-I) is another common treatment option for insomnia. However, the lack of trained practitioners prevents the proper therapeutic outcomes for insomnia patients (Edinger and Sampson, 2003). Insomnia is associated with anxiety disorders, depression, suicide, substance abuse, decreased immune functioning, and cardiovascular diseases (Taylor et al., 2003).

Alternative medicinal approaches can provide a solution to these issues. Herbal medicinal practices including Ayurveda are being explored as a complementary and alternative treatment approach (Gyllenhaal et al., 2000). A United States national survey reported that 4.5% of adults prefer complementary and alternative medicine as a treatment measure for insomnia (Pearson et al., 2006).

Ashwagandha (*Withania somnifera* (L.) Dunal.) is being used in Ayurveda for long. This shrub belongs to the *Solanaceae* family and is popularly known as winter cherry and Indian ginseng (Dafni and Yaniv, 1994; Andallu and Radhika, 2000). Ashwagandha is categorized as "*Rasayana*" (rejuvenator) and is believed to maintain health, rejuvenate the body, and may enhance longevity (Sivarajan and Balachandran, 1994). Significant phytochemical constituents such as alkaloids, steroidal lactones (withanolides, withaferins), saponins, glycol-withanolides are present in Ashwagandha (Shah et al., 2006). This plant material has a wide range of pharmacological beneficial effects including anxiolytic, hypotensive, sedative, immunomodulatory, analgesic, anti-inflammatory, anti-tumor, anabolic, antifungal, hematopoietic, and cardiorespiratory enhancing (Choudhary et al., 2015; Shenoy et al., 2012). The plant parts, especially the root, is also used as an adaptogen and antioxidant (Mishra et al., 2005; Sandhu et al., 2010; Kulkarni and Dhir, 2008; Verma and Kumar, 2011). Ashwagandha root extract can increase memory and cognitive abilities in people with mild cognitive impairment (Choudhary et al., 2017a,b), can improve the sexual function in both males and females (Dongre et al., 2015; Ambiye et al., 2013; Gupta et al., 2013), aids in body weight management in adults and improves an individual's resistance towards stress and anxiety (Choudhary et al., 2017a,b; Chandrasekhar et al., 2012).

The species name *Somnifera* refers to sleep induction in Latin. Hence, for years the herb is having literal evidence for sedation or sleep induction. Animal studies demonstrated that oral administration of Ashwagandha root extract induced sleep in rats (Kumar and Kalonia, 2007, 2008). However, human studies on the effect of Ashwagandha related to insomnia are limited. Therefore, the objective of the present study was to assess the efficacy and safety of Ashwagandha root extract compared to the placebo for insomnia and anxiety. Stratified randomization was used to parallelly understand the impact of the herbal extract on healthy volunteers, and insomnia patients. Sleep onset latency was the primary outcome of the study. Total sleep time, sleep efficiency, total time in bed, and wake after sleep onset were the secondary outcomes of the study. Monitoring and assessment of the outcomes were done using actigraphy, Hamilton anxiety scale (HAM-A), mental alertness on rising, and sleep quality. In this study, safe and efficacious outcomes for the participants used Ashwagandha root extract was expected compared to the placebo.

2. Materials and methods

2.1. Study design

The study was designed as a randomized, double-blind, placebo-controlled, parallel-group stratified comparative clinical study, and it was conducted for 8 weeks. Outcome measurements were conducted at baseline, week 1, week 4, and week 8, respectively. Associated International ethical norms and national ethical guidelines were followed throughout this study. Declaration of Helsinki was strictly obeyed and the ethical clearance for the trial was obtained from the ethics committee of DY Patil Medical college hospital and research center on November 4, 2018 (Reference number: DYP/IEC/04–007/2018). The study was conducted at the D.Y. Patil Medical College and Hospital, Nerul, Navi Mumbai. This study was registered at the Clinical Trials Registry of India (Registration no: CTRI/2019/03/018074). We followed the Consolidated Standards of Reporting Trials (CONSORT) guidelines for designing and reporting the trial outcomes (Schulz et al., 2010). All information related to this study can be accessed from the authors.

2.2. Participants

Participants visiting the outpatient facility of the hospital were recruited for the study. Subjects were considered eligible to be enrolled in the study when the following inclusion criteria were met: (1) subjects should be of either gender, aged between 18 and 50 years, (2) healthy subjects were chosen for Arm-A, (3) For Arm-B, subjects having insomnia were selected. Insomnia was characterized by the diagnosis based on DSM-IV criteria. Patients who usually take more than 30 min to fall asleep and habitually take subjective total sleep time of $\leq$6.5 h per night (for at least 3 nights per week), having daytime complaints associated with disturbed sleep, having habitual bedtime between 8.30 p.m. to midnight, and contained Body Mass Index between 16.5 and 30 kg/sq. m., were chosen. Besides, only those who were able to understand the sleep diary, able to and willing to fill the sleep log diary, and committed to obeying other procedures required by the study protocol, were selected. Subjects who signed informed written consent were considered. Simultaneously, the following exclusion criteria were applied for the study: (1) subjects who had suffered from sleep disorder other than

primary insomnia including restless leg syndrome and sleep apnea, (2) subjects having known clinically significant endocrine, metabolic, hepatic, renal, cardiovascular, gastrointestinal, respiratory, hematological or neurological illnesses, (3) participants having any psychiatric disorders that were judged by the investigator to possibly have any relation to insomnia, (4) participants having record of any substance dependence/abuse in the past one year or having alcohol abuse, (5) those who used tobacco products during night awakenings, (6) those who had history of seizures or significant head trauma, (7) subjects who travelled across four or more time zones or worked on night or rotating shifts in previous 7 days before the study initiation or who had plans to do the same during the study period, (8) subjects who were taking any medication on a regular basis (except for antihypertensives, antidiabetics, lipid lowering agents and drugs for primary cardiovascular prophylaxis), (9) subjects who took any other investigational drug within three months prior to this study, (10) those who could not be relied upon to comply with the test procedures or were unwilling to give informed consent, and (11) pregnant or breast-feeding women or women with positive urinary pregnancy test at screening were also excluded.

2.3. Interventions

After obtaining informed consent from each participant and completing a baseline evaluation, participants who met the inclusion criteria and none of the exclusion criteria were randomized to one of the two treatment groups in both the arms, namely, the intervention group, or the placebo group. The intervention group received the Ashwagandha root extract capsule (KSM-66) manufactured by Ixoreal Biomed Inc., California, USA as the investigational product. The KSM-66 Ashwagandha root extract is a commercially available product. Ashwagandha cultivation requires proper sandy dry soil with a pH of 7.5–8.0 for optimum growth and to have the presence of bioactives like withanolides and other phytochemicals in the root. This extract is manufactured from the roots of ashwagandha plant, using a water-extraction process, without the use of alcohol or any chemical solvents. The processing of the roots is done based on the principles of green chemistry in a current good manufacturing practice (cGMP) certified facility. The herb to extract ratio is 15:1. Every batch of this extract is standardized to a withanolide content of >5% by HPLC. Further, each batch undergoes rigorous quality testing including organoleptic testing, moisture content estimation, microscopic and Petri-grid analysis, microbiological and Ash testing, pH assessment, Aflatoxin testing, and heavy metal analysis, pesticide content analysis, and bioactive analysis. The product used was having the batch number KSM/19/S013 (Mfd: 02/2019, Exp: 01/2022). The Ashwagandha root extract test product was having yellowish powdery appearance. The placebo group received only starch as the placebo capsules that were of the same size, shape, odor, color, and taste as the Ashwagandha capsule. Participants were instructed to have one capsule twice daily with either milk or water for 8 weeks. Each capsule was of 300 mg dosage.

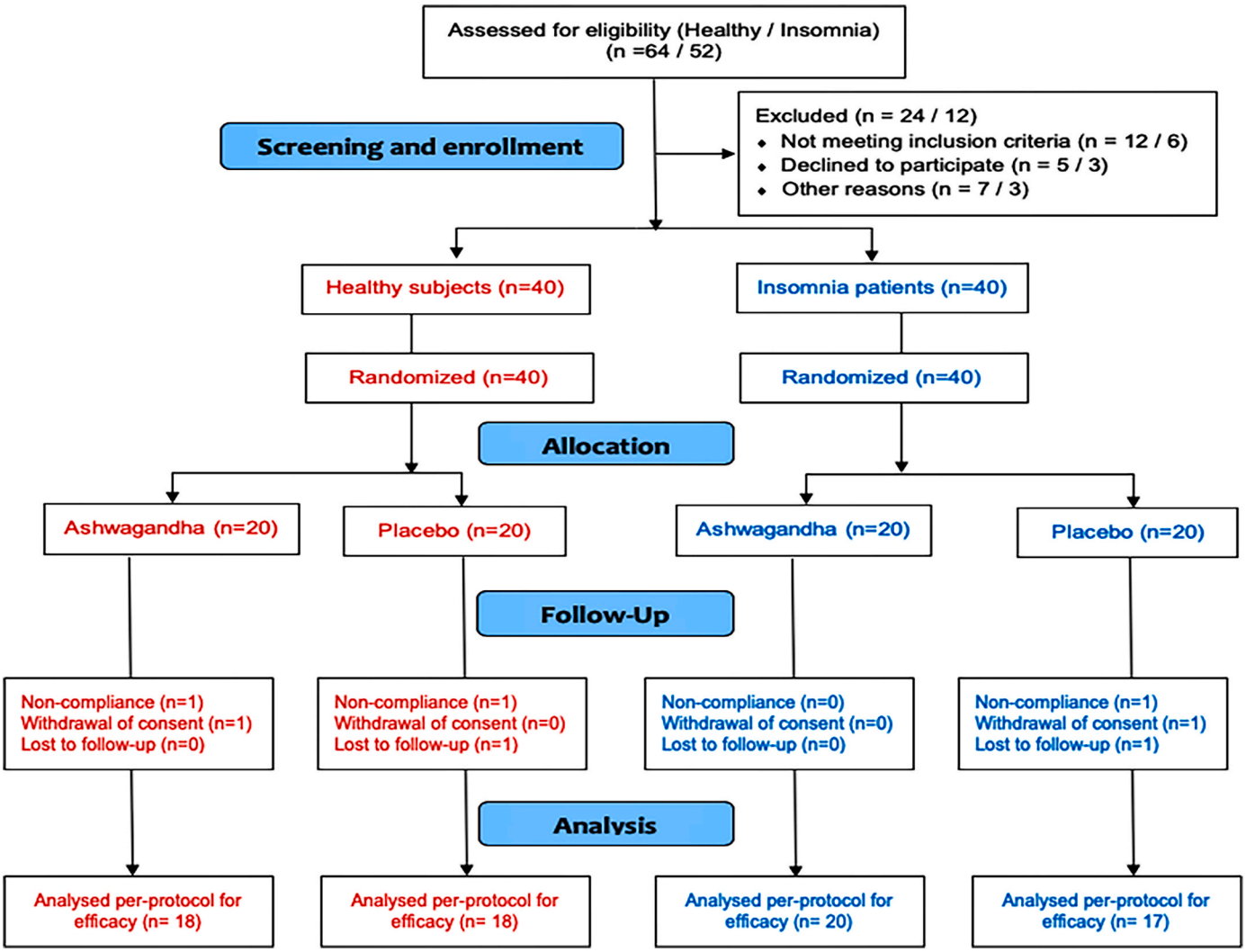

Fig. 1. CONSORT flow diagram representing the screening and enrollment, allocation, follow-up, and analysis conducted on the considered participants.

2.4. Randomization and blinding

Participants were enrolled in two arms for the study as represented in Fig. 1. CONSORT flow diagram. Arm-A comprised of healthy participants and Arm-B comprised of participants diagnosed with insomnia. Stratified randomization was performed for Arm-A and Arm-B separately using a computer-based predetermined randomization block of 40. Each block had subjects randomized to either the Ashwagandha group or the placebo group.

Participants were not aware of their group assignments in the trial. Researchers including statisticians, outcome assessors, and data analysts were all blinded related to the patients' group assignments. The treatment and placebo medication packs were tamper-proof and identical in appearance and weight. The packs were coded to conceal their contents, and the label contained the subject serial number only. The randomization codes were maintained in separate sealed envelopes for each patient. It was allowed to open by the investigator only after the subject was enrolled and received the serial number. During data collection, the research coordinators, the study investigators, and the attending care personnel were not allowed to access the randomization codes and allocations.

2.5. Outcome measures

2.5.1. Sleep parameters-actigraphy

Sleep parameters such as Sleep Onset Latency (SOL), Total Sleep Time (TST), Wake After Sleep Onset (WASO), Total time in bed (TIB) and Sleep efficiency (SE) were assessed in both the Arms, i.e., Arm-A and Arm-B. The sleep parameters were assessed using Actigraphy, which is a non-invasive method of monitoring human rest and activity cycles. All the parameters were measured at baseline, week 4 and week 8.

2.5.1.1. Sleep onset latency. The primary outcome measure of this study was to assess the change in Sleep Onset Latency through actigraphy. Sleep onset latency refers to the duration of time taken to complete the transition from full wakefulness to sleep, generally to the lightest of the non-rapid eye movement sleep stages.

2.5.1.2. Total sleep time. One of the secondary outcome measures of this study was to assess the total sleep time. Total Sleep Time is the amount of actual sleep time in a sleep period. Total sleep time is equal to the total sleep period except for the movement and awake time. Total sleep time refers to the summation of the rapid-eye-movement sleep and non-rapid eye movement sleep in a sleep period.

2.5.1.3. Wake after sleep onset. Wake After Sleep Onset is the amount of time spent awake after sleep has been initiated and before the final awakening. It is an important parameter and refers to the period of wakefulness occurring after defined sleep onset. This parameter measures wakefulness, excluding the wakefulness occurring before sleep onset.

2.5.1.4. Sleep efficiency. Sleep efficiency refers to the percentage of the total time in bed spent in sleep. In other terms, sleep efficiency is the ratio of time spent asleep to the amount of time spent in bed.

2.5.2. Pittsburgh Sleep Quality Index (PSQI)

The Pittsburgh Sleep Quality Index is a seven-component questionnaire that contains 19 questions altogether. The seven components are subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications and daytime dysfunction over the last month (Buysse et al., 1989). This questionnaire was developed from the clinical intuitions and the long experience of the medical practitioners dealing with sleep disorder patients. The questionnaire also contains 5 additional questions for the bed partner or roommate where a secondary observation can be reported. However, as these questions are not considered for scoring as per the guideline, we did not consider these questions for this study. As recommended by the scale developer, all the questions were scored individually. The individual question was scored between 0 and 3 where obtaining a value near 3 represents the negatively extreme outcome on a Likert scale. The sum of the seven component scores or the global score was calculated for each participant. The subjective sleep quality global score varied from 0 to 21. Higher scores indicate worse sleep quality. Assessments were done at baseline, week 4 and week 8 respectively.

2.5.3. Hamilton Anxiety scale-A (HAM-A)

Hamilton Anxiety scale-A, a psychological questionnaire, is one of the first rating scales to measure the severity of perceived anxiety symptoms (Hamilton, 1959). The HAM-A probes 14 parameters and it takes 15–20 min to complete the interview and score the results. Each parameter is defined by a series of symptoms and measures both psychic anxiety and somatic anxiety. Each item is scored on a scale of 0–4, where 0 represents no symptoms present and 4 represents severe symptoms. A total score ranges from 0 to 56, where score less than 17 indicates mild severity, 18–24 indicates mild to moderate severity and 25–30 indicates moderate to severe anxiety.

2.5.4. Mental alertness on rising

This was considered as a secondary outcome measure in this study and it was used to measure the alertness perceived by the patient after waking up in the morning. Mental alertness on rising is generally assessed using a three-point scale.The scoring is done as following: 1 = alert, 2 = slightly drowsy and 3 = extremely drowsy.

2.5.5. Sleep quality

The sleep quality of the subjects was measured using a seven-point sleep quality scale. The scale was used to assess the overall sleep quality as perceived by the subjects after waking up in the morning. The scoring for the sleep quality scale was as follows: 1 = Excellent, 2 = Very good, 3 = Good, 4 = . Fair, 5 = Poor, 6 = Very poor and 7 = Extremely poor. Less score indicates a better quality of sleep.

2.6. Safety

The intervening product used in this study is a botanical with no carcinogenic, mutagenic, teratogenic, or development associated toxic effect. However, the safety of the participants was assessed by physical examinations, vital signs and laboratory tests including heart rate, blood pressure, body weight and routine blood test at baseline, week 1 and week 8.

2.7. Concomitant medication

Strict regulation was maintained for concomitant medications. Therefore, it was decided that this trial should not be combined with any other interventions such as other sleep medications, antipsychotics, antidepressants, and any drugs related to the central nervous system. It was considered acceptable when the patients took other drugs that do not affect the central nervous system before the trial. During the case recording, careful documentation was done for the dosage, duration, and name of any concomitant treatment or medication the patients might have taken. Moreover, it was decided that any other required concomitant medication for the patient would be prescribed at the discretion of the investigator or the attending clinician following the routine clinical practice at the study site.

2.8. Sample size

This being an exploratory study, it was planned to include both healthy subjects and patients diagnosed with insomnia. Thus, a total of

80 subjects were enrolled in the study with 40 healthy subjects in Arm-A and 40 subjects diagnosed with insomnia in Arm-B. Further, the 40 participants in each group were randomly allocated to either Ashwagandha or the placebo group by a 1:1 ratio. The sample size was not calculated based on any statistical assumption. Earlier, we considered a study population of 50 participants (Choudhary et al., 2015). Therefore, we have arrived at an equivalent number of participants in individual groups after the eligibility assessment.

2.9. Data analysis

All data analyses were conducted by statisticians using MedCalc Statistical Software version 19.0.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2019). All the analysis was done on both intent-to-treat (ITT) and per-protocol (PP) datasets. Baseline data for demography and scores were compared between the two groups of Ashwagandha and Placebo. Data normality was tested using the Shapiro-Wilk test. Repeat measures ANOVA was used to compare the treatment effects (Ashwagandha and Placebo) at different periods (Baseline, 1 week, 4 weeks and 8 weeks) of interventions. Non-parametric tests were used for ranking data and non-normal data. Baseline scores were compared to post-treatment scores within each group using the Friedman test followed by post-hoc individual comparisons using the Wilcoxon test. Between groups, comparisons were done using Mann-Whitney 'U' test. Two-Way Analysis of Variance (ANOVA) was used to analyze the effects of treatment (Ashwagandha and Placebo), and clinical conditions (Insomnia and healthy subjects), for sleep actigraphy parameter scores at 4th and 8th week, respectively. The statistical significance level was considered at $p < 0.05$ and 95% confidence interval (CI). 95% CI was used for all the continuous variables.

3. Results

3.1. Participants distribution

A total of 116 subjects (64 healthy subjects and 52 patients with insomnia) were assessed for eligibility. Out of these, 36 subjects were excluded from the study (18 did not meet the inclusion criteria, 8 declined to participate and 10 had other reasons to refuse participation). As a consequence, a total of 80 participants were recruited for the study (Arm-A consisting of 40 healthy subjects and Arm-B consisting of 40 insomnia patients). All the participants were randomly assigned to two groups: the Ashwagandha group and the placebo group. In Arm-A, 2 subjects withdrew from both Ashwagandha and the placebo group. In Arm-B, 3 participants withdrew from the placebo group. There were no missing data in the remaining 73 participants who completed the study. Fig. 1 depicts the flow chart illustrating the inclusion, exclusion, allocation, and analysis of the study participants.

3.2. Demographic characteristics

The baseline demographic characteristics of the participants are presented in Table 1. The mean age of healthy participants in the Ashwagandha group was observed as 35.60 ± 8.74 years and in the placebo group, it was 38.25 ± 7.83 years. Consecutively, the mean age of the participants having insomnia was found to be 38.70 ± 7.15 years in the experimental Ashwagandha group and 35.95 ± 5.49 years in the placebo group. There was no significant difference between the two groups regarding age, BMI, blood pressure (systolic and diastolic), pulse rate, respiratory rate and other baseline parameters ($p > 0.05$).

3.2.1. Sleep parameters assessment outcome

Sleep parameters such as sleep onset latency, total sleep time, wake after sleep onset, total time in bed and sleep efficiency were recorded using an actigraphy device during screening, at baseline, week 4 and at week 8. Assessments were done in both the Arm-A and Arm-B.

In both Arm-A of healthy subjects and Arm-B of insomnia patients, there was a decrease in sleep onset latency and wake after sleep onset (Fig. 2A and Figs. 2B and 3A and Fig. 3B). On the other hand, an increase in total sleep time, total time in bed (Fig. 2C and D) and sleep efficiency (Fig. 3E and F) were noted over the 8-week study in both Ashwagandha and placebo group. The estimated standard error and other statistics on the sleep parameters are presented in Supplementary Table 1.

It was observed that the baseline scores of the sleep parameters were significantly different in both the arms. At baseline, sleep onset latency and wake after sleep onset was higher in insomnia subjects (Arm-B) when compared to the healthy subjects (Arm-A) (Figs. 2 and 3). Total sleep time, total time in bed and sleep efficiency were recorded lower in the Arm-B (Insomnia group) when compared to the Arm-A (Healthy subject group).

All the sleep parameters were subjected to statistical significance analysis for the obtained baseline values and the end of the study results. The repeated measure ANOVA outcome is presented in Table 2.

In the healthy subject group, the obtained p-values for SOL, TST, WASO, TIB, SE were found significant ($p < 0.0001$). A significant outcome was observed for the Insomnia group outcomes as well for these parameters ($p < 0.0001$).

3.3. PSQI assessments outcome

Our observations suggest a decrease in the PSQI scores in both the clinical trial arms (Fig. 4). The mean PSQI score at baseline was recorded as 4.10 ± 0.20 and 9.04 ± 0.29 for the Ashwagandha treatment group in Arm-A and Arm-B, respectively.

At the end of the study, it was noted that the mean PSQI score was changed to 3.19 ± 0.19 and 6.67 ± 0.34, respectively, suggesting improvements in the outcomes (Fig. 4, Supplementary Table 2). The recorded decrease was statistically significant in both the trial arms A and B.

The within the group comparison for the PSQI results for the

Table 1
Demography at baseline in ITT dataset (All randomized, n = 80).

	Healthy (n = 40)				Insomnia (n = 40)				All patients (n = 80)			
	Ashwagandha (n = 20)		Placebo (n = 20)		Ashwagandha (n = 20)		Placebo (n = 20)		Ashwagandha (n = 40)		Placebo (n = 40)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (yrs.)	35.60	8.74	38.25	7.83	38.70	7.15	35.95	5.49	37.15	8.04	37.10	6.78
BMI (kg/sq.m.)	27.26	3.91	26.80	3.36	27.15	3.81	27.74	5.22	27.21	3.81	27.27	4.36
Systolic BP (mm Hg)	118.60	8.90	118.80	7.06	120.70	7.63	122.10	8.22	119.65	8.25	120.45	7.75
Diastolic BP (mm Hg)	77.60	6.28	79.00	6.66	81.20	6.17	80.90	4.18	79.40	6.41	79.95	5.57
Pulse rate (per min.)	17.90	1.77	16.90	1.21	17.50	1.57	17.30	1.49	17.70	1.67	17.10	1.35
Temperature (0F)	98.21	0.15	98.18	0.13	98.14	0.14	98.22	0.12	98.17	0.15	98.20	0.13
Respiratory rate	18.10	2.00	16.90	1.21	17.60	1.79	17.30	1.49	17.85	1.89	17.10	1.35

$p > 0.05$ for all comparisons (Ashwagandha Vs Placebo).

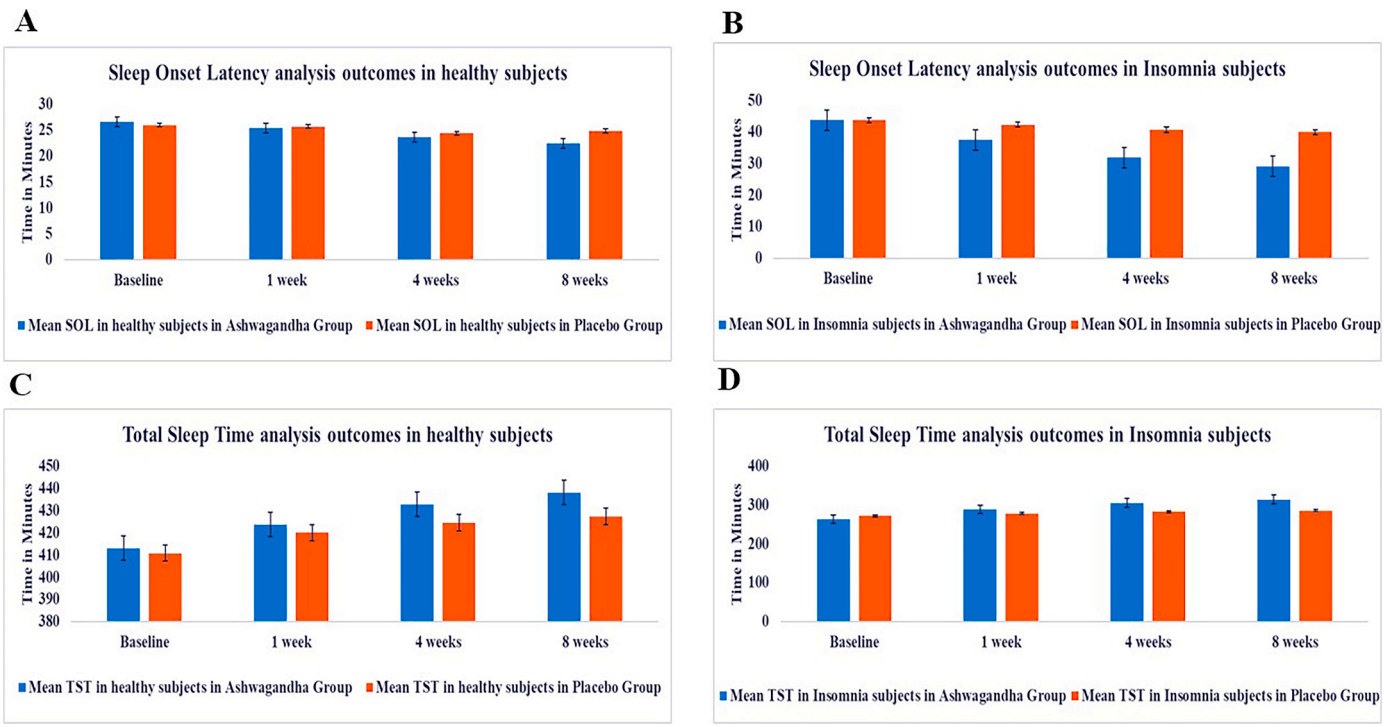

Fig. 2. Outcome representation of Sleep Onset Latency (A and B) and Total Sleep Time analysis (C and D) for the healthy participants and insomnia patients in the PP dataset.

treatment groups (Ashwagandha) both in healthy and Insomnia division was found statistically significant ($p < 0.0001$) (Table 2).

3.4. Hamilton Anxiety scale-A (HAM-A) measurement outcome

The HAM-A assessment was performed at baseline, and at the study week 1, 4 and 8 for both the groups and in Arm-A and Arm-B simultaneously. Among the healthy subjects in Arm-A, the decrease in HAM-A scoring was not found to be much significant when compared to the decrease in the insomnia patient group (Fig. 5A).

In Arm-B, the baseline score was noted as 22.81 ± 0.94 in the Ashwagandha group, and 23.02 ± 1.02 in the placebo group. At the end of the study, the score in the Ashwagandha group was recorded as 15.92 ± 1.00 and 21.09 ± 1.08in the placebo group (Fig. 5B, Supplementary Table 3). The reduction in the values was statistically significant in the Ashwagandha group when compared to the placebo group in Arm-B.

The HAM-A outcomes for the treatment group in the Insomnia division were found statistically significant ($p < 0.0001$). However, for the healthy study population group, it was not significant.

3.5. Mental alertness on rising

As mentioned earlier, mental alertness on rising was assessed at baseline, week 1, week 4, and week 8 in both the study arms, i.e., Arm-A and Arm-B (Fig. 6, Supplementary Table 4).

After 8 weeks of the study, the analysis of the obtained data suggested that there was a significant decrease in the mean score of mental alertness in both the arms in the Ashwagandha supplemented group compared to the placebo group. The mental alertness outcome for the treatment group for the Insomnia patients was found statistically significant ($p < 0.0001$).

3.6. Sleep quality assessment outcome

The obtained outcome of the sleep quality scores in the healthy subjects (Arm-A) at baseline was 2.09 ± 0.22 in the Ashwagandha group and 2.18 ± 0.22 in the placebo group (Fig. 7A). After 8 weeks of the study, the obtained analyzed sleep quality score was 1.43 ± 0.18 in the Ashwagandha group and 1.96 ± 0.18 in the placebo group (Fig. 7A, Supplementary Table 5). Overall, there was a significant decrease in the score values suggesting the improvement in the sleep quality of the healthy participants enrolled in the study. Within the group improvement evaluation for the sleep quality displayed statistically significant outcomes ($p < 0.0001$) for both the Ashwagandha group, in the healthy study population and the Insomnia study group (Table 2).

Simultaneously, in the insomnia group, the baseline scores in both the Ashwagandha and the placebo group were 5.74 ± 0.19 and 5.72 ± 0.21, respectively (Fig. 7B). A significant decrease in the scores was observed by the end of the study and the score in the Ashwagandha group was recorded as 3.14 ± 0.23, and in the placebo group, it was 5.01 ± 0.25, respectively (Fig. 7B). The sleep quality increased significantly in both the arms, but comparatively the Ashwagandha treated group displayed better outcomes, especially for the patients having insomnia.

The repeat-measure ANOVA conducted for the insomnia patients of Arm-B suggested that significant improvement occurred for the important parameters such as sleep onset latency (p 0.013), anxiety (HAM-A) ($p < 0.05$), mental alertness of the insomnia patients (p 0.01), and the sleep quality ($p < 0.05$).

The outcome of the two-way ANOVA represented significant outcomes for the sleep parameters. The most significant outcome was observed for sleep onset latency ($p < 0.0001$) and sleep efficiency ($p < 0.0001$), followed by TST ($p < 0.002$) and WASO($p < 0.040$). However, for TIB, the ANOVA result was not significant ($p < 0.214$).

3.7. Concomitant medication outcome

It was decided to take strict notes on the concomitant medications and it should be provided to the participants of insomnia, if required, after proper evaluation. Interestingly, none of the subjects received any concomitant medication during the study period. This helped the study to avoid any unnecessary biased outcomes.

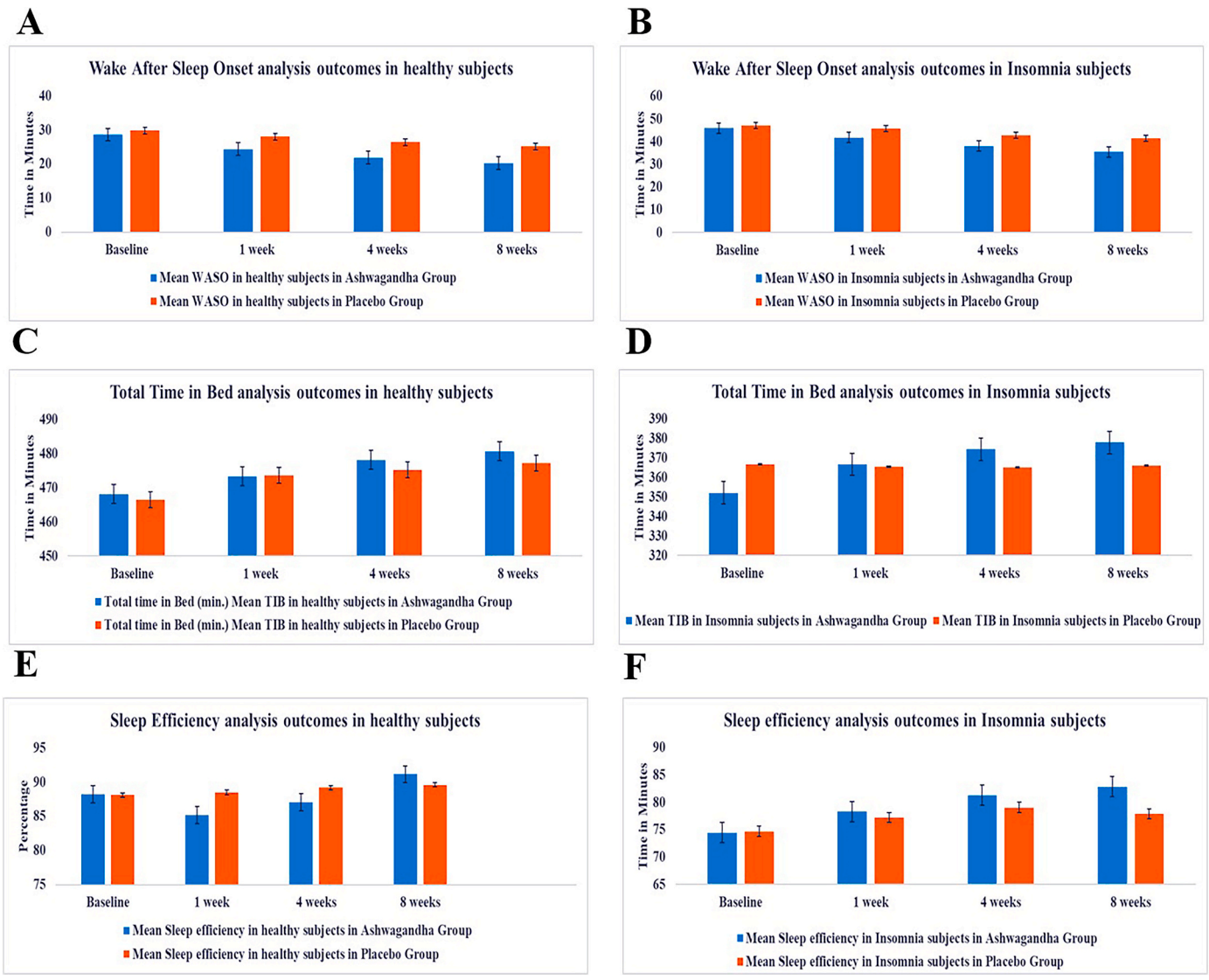

Fig. 3. Outcome representation of the sleep parameters including WASO, TIB, and SE in healthy and insomnia subjects in the PP dataset.

Table 2
Comparative representation of the baseline and the respective end of the study (Within the group) p-values and F values from repeat measure ANOVA for the Healthy subject group, and Insomnia group.

	Healthy subjects				Insomnia subjects			
	Ashwagandha		Placebo		Ashwagandha		Placebo	
	F	p	F	p	F	p	F	p
SOL	84.771	<0.0001	22.261	<0.0001	105.942	<0.0001	12.473	<0.0001
TST	173.801	<0.0001	65.205	<0.0001	222.010	<0.0001	122.218	<0.0001
WASO	52.397	<0.0001	61.663	<0.0001	120.679	<0.0001	50.642	<0.0001
TIB	20.668	<0.0001	21.208	<0.0001	83.013	<0.0001	0.125	0.945
SE	11.723	<0.0001	172.375	<0.0001	239.112	<0.0001	12.939	<0.0001
PSQI	14.414	<0.0001	2.532	0.094	88.507	<0.0001	45.147	<0.0001
HAM-A	0.000	1.000	1.466	0.243	97.550	<0.0001	19.052	<0.0001
Mental Alert	4.857	0.005	5.255	0.003	26.238	<0.0001	3.168	0.033
Sleep Quality	15.553	<0.0001	2.990	0.039	126.465	<0.0001	12.732	<0.0001

3.8. Adverse events reports

In each step of the assessment, at week 1, week 4, and week 8 at the end of the study, every participant was rigorously questioned regarding any kind of adverse event they might have experienced. Till the last data recording, no adverse events were reported in the study.

4. Discussion

The effect of Ashwagandha was evaluated on the quality of sleep in healthy subjects and insomnia patients in the present study. The analysis outcomes were compared over 8 weeks to the placebo groups. Stratified randomization was opted due to the presence of defined subject groups

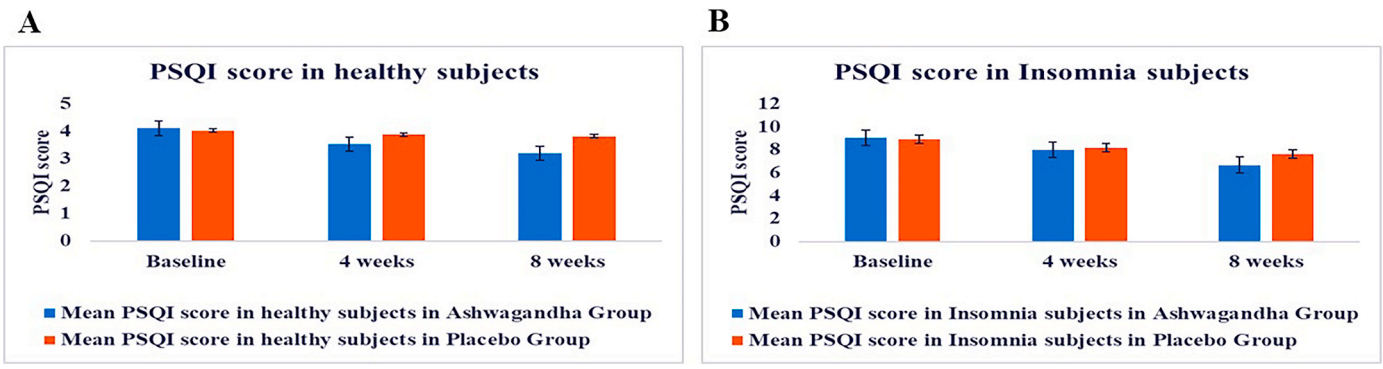

Fig. 4. Presentation of the recorded mean PSQI evaluation scores at baseline, week 4 and week 8, for the healthy and insomnia subjects in the PP dataset.

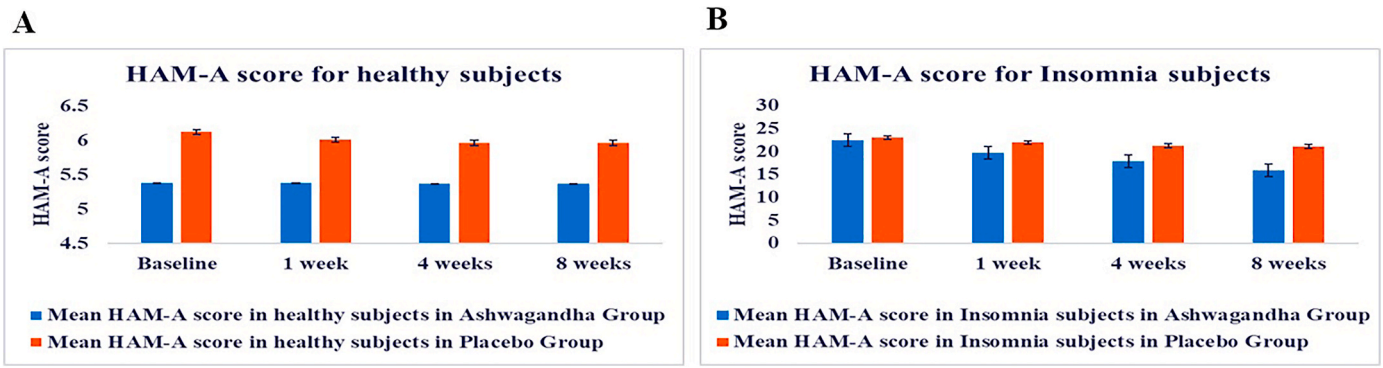

Fig. 5. Recorded HAM-A questionnaire assessment outcome scores at baseline, week 1, week 4, and week 8 for the healthy and insomnia subjects in the PP dataset.

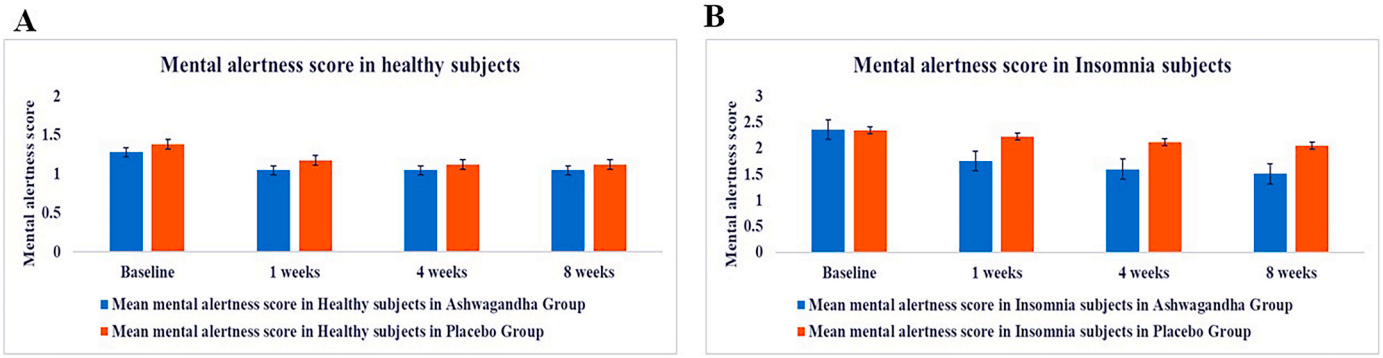

Fig. 6. Mental alertness scoring outcomes for the healthy subjects and insomnia patients in the PP dataset recorded at the baseline, week 1, week 4, and week 8.

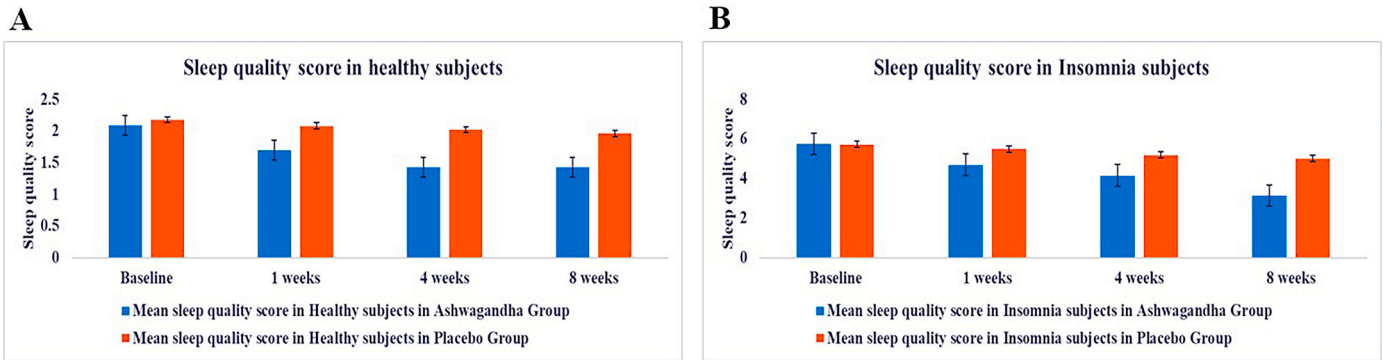

Fig. 7. The outcome of the sleep quality assessment in the healthy and insomnia subjects in the PP dataset.

before the allocation.

Insomnia is affecting the global population of different age groups, specifically the elderly populations. Recent studies and surveys suggested that insomnia is on the rise in the younger and working populations as well. Reports of insomnia were frequent in various medical centers (Buysse, 2013). Sleep disorders and insomnia can affect the quality of life of an individual and can cause physical and psychological health problems. Available medications failed to improve the conditions either due to drug addiction or serious adverse effects, especially in the elderly population (Schroeck et al., 2016).

The sleep physiology entails three major vigilance states, namely, wake state, slow-wave or non-rapid eye movement sleep, and rapid eye movement sleep. The arousal state or wakefulness is managed by the activated ascending reticular activating system (ARAS), pontine brainstem, locus coeruleus (LC), dorsal raphe nucleus (Anaclet et al., 2014), and medial parabrachial nucleus through specific neurochemicals. Anti-GABAergic activity and the thalamocortical network are attributed to maintaining wakefulness and arousal (Fort et al., 2009). On the contrary, the gradual inhibition of such neuronal activity, mostly by gamma-aminobutyric acid (GABA), induces sleep. The process involves activation of the slow-wave neurons through the circadian clock present at the suprachiasmatic nucleus along with the reduction of adenosine quantity. Reports suggest that GABAergic neurons present at the ventrolateral preoptic nucleus, reticular thalamic nuclei and in other locations become activated to induce slow-wave sleep (Luppi and Fort, 2019). Besides, the activity and interaction of GABAergic and glutamatergic neurons in the brainstem and posterior neurons promote the rapid eye movement sleep.

The pharmacological action of the hypnotic or soporific drugs in inducing sleep predominantly include physiological alteration in the neurotransmitter gated ion channels, reduction in cerebral metabolism rate, and GABA mediated activity. The benzodiazepines function through binding to a distinct benzodiazepine binding site present between the α and γ-subunits of the $GABA_A$ receptor (Winsky-Sommerer, 2009). This increases the chloride influx through the ionotropic receptor causing hyperpolarization of the postsynaptic membrane (Rihel and Schier, 2013). An inhibitory effect is triggered through this failing event of a successful action potential generation. Apart from benzodiazepines, the $GABA_A$ receptor site serves as an allosteric site for several other drugs (Szabadi, 2006). Such pharmacological treatments with benzodiazepines, antihistamine medications, melatonin, and non-pharmacological treatments, such as cognitive behavior therapy (CBT) and relaxation therapy are used for the treatment and management of sleep disorders. These treatments have certain side-effects (Sasaki et al., 2017; Borchert et al., 2019), hence, alternative solutions are warranted with specific attention to the herbal therapies.

Over the centuries, Ashwagandha has been established as a multipurpose valuable herb that could be used as a general rejuvenator and in specific ailments. Ashwagandha is known as an excellent sleep inducer for long, but the clinical evidence is limited. In the Ayurvedic medicine system, Ashwagandha (*Withania somnifera* (L.) Dunal.) is known for its low or non-toxic effect. Many pharmacological studies indicate that Ashwagandha is used to treat bronchitis, asthma, ulcer, cancer, emaciation, insomnia, and senile dementia (Mishra et al., 2005). *Withania somnifera* (L.) Dunal. is tested through different preclinical and clinical trials for diabetes, immunomodulation, Parkinson's disease, and other conditions. It is also useful as antioxidant, abortifacient, antibiotic, aphrodisiac, diuretic and sedative (Kulkarni and Dhir, 2008; Bone, 1996).

Ashwagandha was reported to be an effective sleep promoter in Ayurvedic medicine (Bobade, 2019). Methanolic extract of Withania root displayed antagonistic activity for the binding of effective GABA receptor antagonists that-butylbicyclophosphorothionate (TBPS) and [3H]GABA (Mehta et al., 1991). Complete inhibition was attained with 1 mg of the extract. Functional analysis revealed that the root extract was able to enhance the chloride influx in the absence of GABA in neurons. Kumar and Kalonia (2008) reported that Ashwagandha can induce sleep through GABAergic activity in sleep-deprived rats. Moreover, Ashwagandha root extract was successful in reducing sleep latency, waking, while improving the non-rapid eye movement sleep, and total sleep in sleep-deprived Wister rats. Similar studies conducted in mice (Kulkarni et al., 2008; Bhattarai et al., 2010) neurons established the GABAergic activity of Ashwagandha through $GABA_A$ receptors. Recently, a clinical study (Langade et al., 2019) reported the effective outcomes in insomnia condition and anxiety assessed through actigraphy with the similar parameters considered in the present study.

To address insomnia, various equivalent alternative approaches have been attempted so far that include yoga, aromatherapy, acupuncture, and cognitive behavior therapy. Many studies reported the benefit of long-term yoga on insomnia. Banker et al. (Bankar et al., 2013) reported that long-term yoga exerts a positive effect on sleep quality improvement, and quality of life evidenced through reduced PSQI score and improvement in QoL scores. Similarly, chronic insomnia was attempted to cure with yoga and significant improvement was observed for SE, TST, TWT, SOL, and WASO (Khalsa, 2004). However, such therapy cannot be opted, for instance, aged patients, patients with mild physical problems. Moreover, the scientific evidence is limited for such therapy. Also, prolonged and extensive physical efforts are required which may not be suitable for all. Aromatherapy was attempted on women college students that reflected the beneficiary effect of Lavender Aromatherapy on the severity of insomnia, length of time taken to fall asleep, and self-satisfaction with sleep (Lee and Lee, 2006). Similarly, another study reported the combined benefit of aromatherapy and massage on the sleep quality of the nurses (Chang et al., 2017). However, such a process is highly time-consuming and is not equivalently effective to all. Acupuncture is used in the traditional Chinese medicine system and also reported for insomnia. PSQI, insomnia severity index, actigraphy parameters, was proposed in a study protocol to be conducted in women (Li et al., 2019), however, the process of therapy and generalized scientific evidence are absent.

The results of this study showed that the use of Ashwagandha root extract was considerably effective in both healthy subjects and patients with insomnia. Ashwagandha root extract supplementation significantly improved different components of sleep quality in comparison to the placebo used. It was observed at the end of the study that sleep onset latency, wake after sleep onset, and total PSQI scores, decreased significantly in the Ashwagandha supplemented intervention group. Besides, sleep efficiency, total sleep time, mental alertness on rising and sleep quality increased in the intervention group. To our knowledge, the present study is the first clinical trial conducted to assess the effect of Ashwagandha root extract on sleep quality in both healthy adults and patients with insomnia. Although, scientific understanding of herbal therapies has progressed substantially, and they have been used to treat insomnia worldwide (Matthew et al., 2013).

5. Limitations

The present study provided valuable information for considering Ashwagandha root extract as an alternative therapeutic component for insomnia. However, a large multi-centric study with a diverse population can generalize the impact of the present findings. The present study lacks the scope of herb-drug and herb-herb interaction as the included insomnia patients were not using any other medication. The study protocol was developed to evaluate the effect of placebo and the Ashwagandha only, no comparison was done with any existing drug considering the aim of the study, i.e., safety and efficacy of the supplement only.

6. Conclusions

Insomnia is one of the leading global health problems due to our present stressful lifestyle. The presently available medications are not

sufficient to counter the situation and have several restrictions of use such as demographic issues like age, clinical issues such as the existence of specific conditions like diabetes, and hypertension. Hence, a solution in the form of an alternative supplement may become helpful. Ashwagandha is having a tremendous reputation as a somatogenic herb in the Indian traditional Ayurvedic medicine system. However, modern scientific and clinical evidence is warranted to evaluate the safety, efficacy, mode of action of this potent herbal adaptogen.

The current study results demonstrated significant improvements in sleep parameters, PSQI, mental alertness, and anxiety parameters. The results were found to be statistically significant in comparison to the placebo. Therefore, the outcomes are suggesting that Ashwagandha root extract has an admirable effect in improving sleep and relegating anxiety among both healthy and subjects diagnosed with insomnia.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgment

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Glossary/Abbreviations

ANOVA	Analysis of variance
ARAS	Ascending Reticular Activating System
BMI	Body Mass Index
BP	Blood pressure
CBT	cognitive behavior therapy
CBT-I	Cognitive-behavioral therapy for Insomnia
cGMP	Current Good Manufacturing Practice
CONSORT	Consolidated Standards of Reporting Trials
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition
GABA	Gamma-Amino Butyric Acid
$GABA_A$	Gamma-Amino Butyric Acid Receptor A
HAM-A	Hamilton Anxiety scale-A
HPLC	High Performance Liquid Chromatography
ITT	Intent-To-Treat
KSM-66	A commercial product with Ashwagandha root extract
LC	Locus Coeruleus
PP	Per-Protocol
PSG	Polysomnography
PSQI	Pittsburgh Sleep Quality Index
SD	Standard deviation
SE	Sleep Efficiency
SOL	Sleep Onset Latency
TBPS	Tert-ButylbicycloPhosphorothionate
TIB	Total Time in Bed
TST	Total Sleep Time
WASO	Wake After Sleep Onset

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2020.113276.

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