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EVALUATION OF EFFICACY AND SAFETY OF "TEST DRUG" IN PATIENTS SUFFERING FROM PRIMARY INSOMNIA- A RANDOMIZED, DOUBLE BLIND, PLACEBO CONTROLLED, COMPARATIVE, INTERVENTIONAL, MULTI-CENTRIC, PROSPECTIVE, CLINICAL STUDY

Dr. Vinod Kuber[1], Gayatri Ganu[2]*, Dr. Gunwant Yeola[3] and Dr. Narendra Mundhe[4]

[1]Sava Healthcare Ltd, Research and Development Center, MIDC Chinchwad, 411019.
[2]Vigilant Healthcare, 101, Moti Square, Bramhan Ali, Rajgurunagar, Pune – 410505.
[3]Dr. D. Y. Patil College of Ayurved and Research Centre, Sant Tukaram, Nagar, Pimpri- Chinchwad, Pune, Maharashtra – 411018.
[4]KVTR Ayurvedic Collage and Hospital, Boradi, Tal – Shirpur, Dist-Dhule – 425428.

***Corresponding Author: Dr. Gayatri Ganu**

Vigilant Healthcare, 101, Moti Square, Bramhan Ali, Rajgurunagar, Pune - 410505.

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ABSTRACT

Background: "Test drug", a polyherbal formulation developed by Sava Healthcare Ltd, for management of primary insomnia. **Objective:** The main objective of the study was to evaluate efficacy and safety of "Test drug" in comparison to placebo in patients suffering from primary insomnia. **Materials and Methods:** Randomized, double blind, placebo controlled, comparative, interventional, multi-centric, prospective, clinical Study. Patients were randomly assigned to drug or placebo groups and advised to consume 2 tablets after night meal for 28 days. Medication was stopped from 28th to 35th day to check rebound insomnia. Student paired and unpaired t test s, Mann Whitney U test, Chi Square tests were used for data analysis. **Results:** The mean total sleep time and sleep efficiency were significantly improved in drug Test drug compared to placebo. Test drug tablet was significantly effective in reducing time to sleep onset, total number of awakenings, wake time after sleep onset and severity of insomnia. Sleep quality was also significantly improved. Stoppage of treatment for seven days did not show rebound of insomnia. No significant post treatment change in any of the lab investigations was observed in both the groups. **Conclusion:** Test drug is safe and effective for the treatment of primary insomnia without rebound insomnia effect.

KEYWORDS: Test drug, Tablet, Primary Insomnia, Placebo, Randomized, Double Blind.

1. INTRODUCTION

Insomnia is one of the most frequent sleep disorder, it is estimated that around 30% of worldwide population suffer from insomnia.[1-5] Insomnia is categorized into primary and secondary. Primary insomnia is defined as difficulty initiating or maintaining sleep for a minimum of one month. Secondary insomnia may occur as a result of medical, psychiatric, environmental, behavioral, or drug side effects.[6] Chronic insomnia affects quality of life, increases risk of psychiatric and substance use disorder, and further deteriorates the health[7-9] Patients suffering from insomnia show tiredness, daytime sleep, low energy levels reduced capacity to concentrate and perform, irritability and reduced ability to enjoy life. Insomnia also leads to increased absentism from work and greater risks of accidents.[10-11] Insomnia is burden at a personal and societal level.

Drugs currently used for insomnia include benzodiazepine receptor agonists (BzRAs), the melatonin-receptor agonist ramelteon, and the histamine-1 antagonist doxepin, sedating antidepressants, and antipsychotics. However, a number of problems are associated with chronic hypnotic medications use such as psychological dependence and tolerance, reduced daytime functioning, poor sleep quality, and rebound insomnia on withdrawal from the medication. There are reports of leucopenia, thrombocytopenia, and increased liver enzymes with use of doxepin; dizziness, dry mouth, and nausea with trimipramine; and daytime somnolence, dizziness, and weight gain with mirtazapine. Carryover sedation among primary insomniacs taking 50 mg of trazodone is also reported. In studies of depressed patients, side effects of trazodone include orthostatic hypotension, priapism, and cardiac arrhythmias and conduction abnormalities.[12-14]

"Test drug" is developed by Sava Healthcare Ltd for Savesta Lifesciences INC. It is a polyherbal formulation of well-known ayurvedic ingredients. The ingredients

used are individually known for their sleep promoting, anxiolytic actions.[14-25] Insomnia has multifactorial etiology and hence a combination of the ingredients acting by different way would be an effective cure, hence a combination of the ingredients was decided for evaluation. A Randomized, Double Blind, Placebo Controlled, Comparative, Interventional, Multi-centric, Prospective, Clinical Study" was therefore carried out.

2. METHODOLOGY

2.1 Study design

This was a randomized, double blind, placebo controlled, comparative, interventional, multi-centric, prospective, clinical study.

2.2 Sample Size

Sample size calculation was derived based on primary end point i.e. change in patient reported total sleep time. Based on the assumptions that mean change in total sleep time in placebo group would be -0.02 with standard deviation of 0.3 and mean change in total sleep time in test group would be 0.6 with standard deviation of 0.7, with desired precision of 5%, a total of 50 completed cases (25 in each group) were needed to assess the study objective at 80% power and 5% level of significance.

2.3 Study objectives

Objective of the study was to compare drug with the placebo. Primary objective was comparison of total sleep time and sleep efficiency (Total sleep time/ time in bed*100) from patient diary. Secondary objectives were assessing the time to sleep onset, number of awakenings, wake time after sleep onset (WASO), severity of insomnia, daytime fatigue, daytime mood, ability to function at work, concentration and memory, rebound of insomnia on day 35, quality of sleep and global overall improvement by investigator and by patient at the end of the study treatment. Safety and tolerability, adverse events/ adverse drug reactions and laboratory parameters were also studied.

2.4 Subject selection

61 subjects diagnosed with primary insomnia having insomnia severity index more than 7 and less than 21 between age 21-65 were included in the study. Subjects having history or diagnosis of another sleep disorder, difficulty in sleeping due to a medical condition, any neurological disorder, bipolar disorder, psychotic disorder, or posttraumatic stress disorder, or current psychiatric disorder that requires medication or on-going depression and generalized anxiety disorder, history of hepatitis B and/ or C, clinically significant cardiovascular disorder were excluded from the study. Also subjects having history of substance abuse or dependence, habit of smoking cigarette were excluded from study. Subjects having history of any malignancy ≤5 years prior to signing informed consent, or known to have hypersensitivity to any of the ingredients of "Test drug" were excluded from the study. Precautions were taken not to recruit the subjects belonging to possible vulnerable groups.

2.5 Investigational drug

The investigational product "Test drug" is approved by State FDA as SERENE Tablet. The composition of the product is given in the Table 1.

Table 1: Composition of "Test Drug" tablet.

Sr.No	Ingredients	Latin Name	Quantity (mg)
1.	Tagar extract	*Valeriana wallichii*	75
2.	Ashvagandha Extract	*Withania somnifera*	150
3.	Shankhapushpi Extract	*Convolvulus pluricaulis*	120
4.	Brahmi Extract	*Bacopa monierri*	70
5.	Jatamansi Extract	*Nardostachys jatamansi*	70
6.	Jatiphala Extract	*Myristica fragrans*	15

2.6 Study procedure

The study was conducted at four sites vis. Site-1: KVTR Ayurvedic Collage and Hospital, Boradi, Tal-Shirpur, Dist- Dhule- 425428; Site-2: Dr. D. Y. Patil College of Ayurved and Research Centre, Sant Tukaram Nagar, Pimpri- Chinchwad, Pune- 18; Site-3: Dhanvantari Clinic, Block 4-5, Omkar Park, Phase-1, Near Rajmudra Society, Behind Bharti Vidyapeeth, Dhanakwadi, Pune-43 and Site-4: Sunad Ayurved Chinchwad, Wolf Colony, Chinchwad- 411033.

There were 7 study visits as screening Visit (Up to Day-14), baseline Visit (Day 0), visit 1 (Day 7), visit 2 (Day 14), visit 3 (Day 21), visit 4 (Day 28), visit 5 (Day 35). Subjects were allowed to come for follow up either 3 days prior or after the scheduled follow up visit, provided subject continued the given treatment.

On screening visit, a written informed consent was obtained from subject for his/her participation in the study. Subject's Dosha Prakriti Parikshan was done. Subject underwent physical and systemic examinations. Subject's medical and surgical history was taken. Subjects not having depression and generalized anxiety disorder were called next day empty stomach for laboratory investigations.

Subject's investigations [CBC, ESR, Hb%, BSL-F, Liver function test s, Renal function test s, Lipid profile, urine examinations, HIV test, UPT (only for fertile females)] were done. Subject's chest x-ray PA view (to rule out active tuberculosis) and ECG (to rule out arrhythmia and recent ischemia) were done. Subjects were advised to refrain from any conventional treatment for primary insomnia. Also subjects were advised to refrain from any

Nutraceutical, Ayurvedic, homeopathic, Siddha, Unani etc. treatment for primary insomnia. Throughout study period, cognitive behavioral therapy for insomnia (CBT-I) was advised to subjects in both the groups. CBT-I consists of five treatment components: sleep education, stimulus control, sleep restriction, relaxation techniques, and cognitive therapy. A screening window of up to 14 days was kept, in case if there was delay in availability of test s reports or in case few test s needed to be repeated. Subjects were called on baseline visit (day 0).

On baseline visit, subjects meeting the inclusion criteria were recruited. Subjects were either randomized to drug group or placebo group in 1:1 ratio. Subjects underwent general and systemic examinations. A sleep diary was given to subject to record time to sleep onset, total sleep time, number of awakenings, wake time after sleep onset (WASO: is defined as total awakening time from falling asleep to final awakening) and sleep efficiency. Subjects were trained how to fill sleep diaries and were instructed to do this every morning. Subjects were evaluated to check whether sleep problem interferes with his/her daily functioning such as daytime mood, ability to function at work, concentration and memory on graded scale (0= Not at all Interfering, 1= A Little, 2= somewhat, 3= Much, 4= Very Much Interfering). Daytime fatigue (if any) was evaluated using fatigue severity scale; quality of sleep was evaluated using Pittsburgh Sleep Quality Index (PSQI). Subjects were advised not to consume alcohol, caffeine, and nicotine during the study period. As per computer generated randomization list, subjects either received "Test drug" or placebo. Subjects were given medication packed in HDPE bottle (each containing 30 tablets). Subjects were advised to take given medication in a dose of 2 tablets orally after evening/night meal with water for 28 days. After 28 days, subjects were advised to stop taking study medication and come for follow up after 7 days to check rebound insomnia. Subjects who continuously missed dosing for >3 consecutive days or total missed doses >6 days during the study period were treated as drop outs.

On every follow up visit, subjects underwent general and systemic examinations. Filled in sleep diary was collected and new diary was given. Subjects were evaluated to check whether sleep problem interferes with his/her daily functioning such as daytime mood, ability to function at work, concentration and memory on graded scale. Subject's daytime fatigue (if any) was evaluated using fatigue severity scale. On day 14, Subject's severity of insomnia was evaluated using Insomnia Severity index.

On day 28, subject's global evaluation for overall improvement and Investigator's global evaluation for overall improvement were done. Subject's severity of insomnia was evaluated. Tolerability of the study drugs was assessed by the investigator and patient at the end of the study. All the subjects were closely monitored for any adverse events/ adverse drug reactions from baseline visit till the end of the study. On day 28, subject's investigations and ECG was done. On day 35, filled in sleep diary was collected from subjects and data were noted in the CRF. Subjects were evaluated to check whether sleep problem interferes with his/her daily functioning such as daytime mood, ability to function at work, concentration and memory on graded scale. Subject's daytime fatigue (if any) was evaluated using fatigue severity scale. Subjects were advised to take investigator's advice for further treatment.

2.7 Ethics

The study was initiated only after a written approval obtained from Independent/Institutional Ethics Committee (IEC). The study was conducted as per approved protocol and as per Good Clinical Practices (GCP) guidelines given by AYUSH in March 2013. After getting approval from the ethics committee, the study was registered on website of Clinical Trial Registry of India (CTRI). The CTRI number of the study is CTRI/2018/04/013035, registered on 04/04/2018. Subjects were enrolled in the study only after registration of the study on CTRI website.

2.8 Statistics

All statistical data analysis were performed using statistical software SPSS version 10.0. The data for primary efficacy variables were analyzed by using student paired and unpaired t test. Other secondary efficacy variables i.e. change in severity of insomnia, change in daytime fatigue assessed on Fatigue Severity Scale (FSS), Change in Daytime mood, ability to function at work, concentration and memory assessed on a graded scale, Change in quality of sleep assessed on Pittsburgh Sleep Quality Index (PSQI) and were analyzed by Wilcoxon sign rank and Mann Whitney U test. Other variables like assessment of rebound insomnia, global assessment for overall improvement by investigator and by patient, tolerability of study drugs were analyzed by Chi Square test. In this study all P values were reported base on two-sided test and these statistical tests were interpreted at 5% level of significance.

3. RESULTS

A total of 61 subjects suffering from primary insomnia were screened for recruitment in the study. 01 subject did not fulfil the inclusion /exclusion criteria and hence was not enrolled in the study. Total 60 subjects were recruited in the study. All the 60 recruited subjects (30 in each group) completed the study.

3.1 Demographic details

Out of 60 completed subjects, 15 (50.0%) were males, while 15 (50.0%) were females in Test drug group while in placebo group, 13 (43.3%) were males, while 17 (56.7%) were females. The mean age of subjects in Test drug Group was 42.27+11.92 years and mean age of subjects in placebo group was 40.40 + 13.61 years. The

comparison between two groups was found to be statistically insignificant as mentioned in Table 2.

Table 2: Demographic details.

Parameters	Test Drug	Placebo
No. of Cases	**30**	**30**
@ Age (yrs.)		
Mean	42.27	40.40
SD	11.92	13.61
Range	23.00 – 64.00 yrs	21.00 – 65.00 yrs
#Sex (%)		
Male	15 (50.0)	13 (43.3)
Female	15 (50.0)	17 (56.7)

@By Student t Test P > 0.05, Not Significant # By Chi – Square Test

3.2 Efficacy Assessments

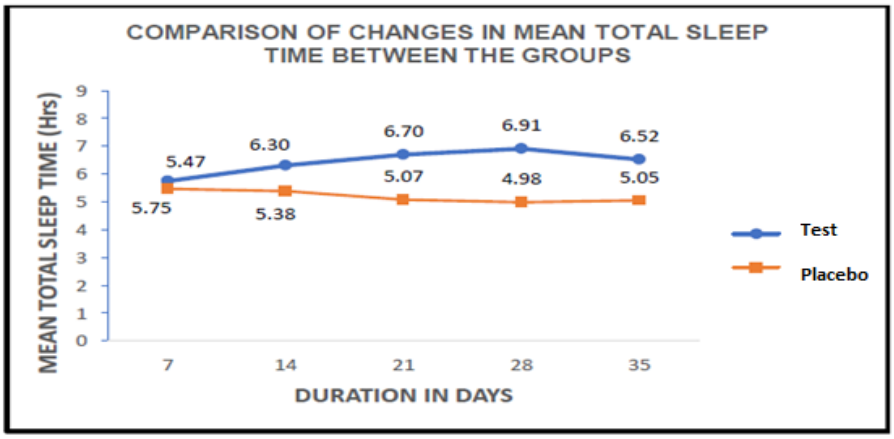
3.2.1 Total sleep time between the groups

In test drug group, total sleep time significantly increased from 5.75 ± 0.84 hrs on day 7 to 6.91 ± 0.79 on day 28, this reduced slightly to 6.52 ± 0.68 on day 35, but was significantly higher than day 7. In placebo group, total sleep time significantly decreased from 5.47 ± 0.76 hrs, on day 7 to 4.98 ± 0.87 on day 28, which slightly increased to 5.05 ± 0.82 on day 35, but was significantly lower than day 7. Test drug performed significantly better than placebo on day 14, day 21, day 28 and day 35. The details are presented in Table 3 and Graph 1.

Table 3: Total sleep time between the groups.

Duration (Days)	Mean total sleep time (Hrs) (X±SD)		P value
	Test Drug (N = 30)	Placebo (N = 30)	
07	5.75 ± 0.84	5.47 ± 0.76	0.181 (NS)
14	6.30 ± 0.92	5.38 ± 0.87	*0.001
21	6.70 ± 0.78	5.07 ± 0.87	*0.001
28	6.91 ± 0.79	4.98 ± 0.87	*0.001
35	6.52 ± 0.68	5.05 ± 0.82	*0.001

By Student t Test *Significant NS = Not Significant



Graph 1: Total sleep time between the groups.

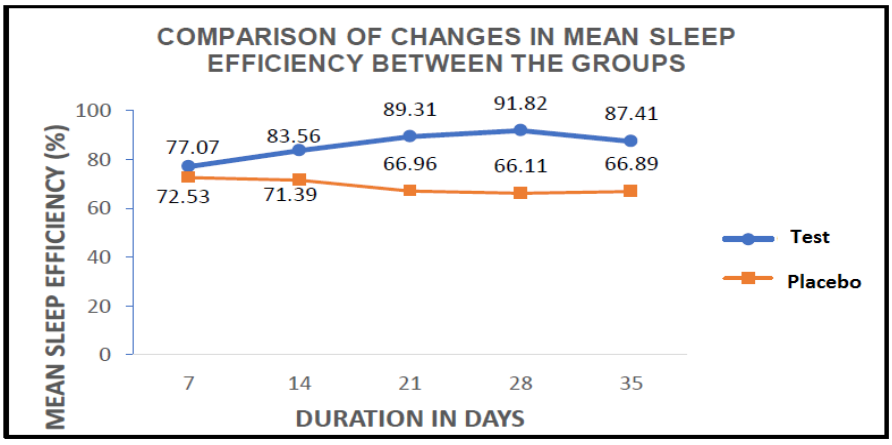
3.2.2 Sleep efficiency between the groups

In test drug group, the mean sleep efficiency significantly increased from 77.07 ± 9.39, on day 7 to 91.82 ± 5.99 on day 28, this reduced slightly to 87.41 ± 5.15.on day 35, but was significantly higher than day 7. In placebo group, the mean sleep efficiency significantly reduced from 72.53 ± 7.32 on day 7 to 66.11 ± 6.82 on day 28, which slightly increased to 66.89 ± 7.17 on day 35, but was significantly reduced from day 7. When compared between the groups, Test drug performed significantly better than placebo on day 14, day 21, day 28 and day 35. The details are presented in Table 4 and Graph 2.

Table 4: Sleep efficiency between the groups.

Duration (Days)	Mean Sleep Efficiency (%) (X±SD)		P value
	Test Drug (N = 30)	Placebo (N = 30)	
07	77.07 ± 9.39	72.53 ± 7.32	*0.041
14	83.56 ± 8.36	71.39 ± 8.55	*0.007
21	89.31 ± 5.99	66.96 ± 7.09	*0.001
28	91.82 ± 5.99	66.11 ± 6.82	*0.001
35	87.41 ± 5.15	66.89 ± 7.17	*0.001

By Student t Test *Significant NS = Not Significant



Graph 2: Mean Sleep Efficiency between the groups.

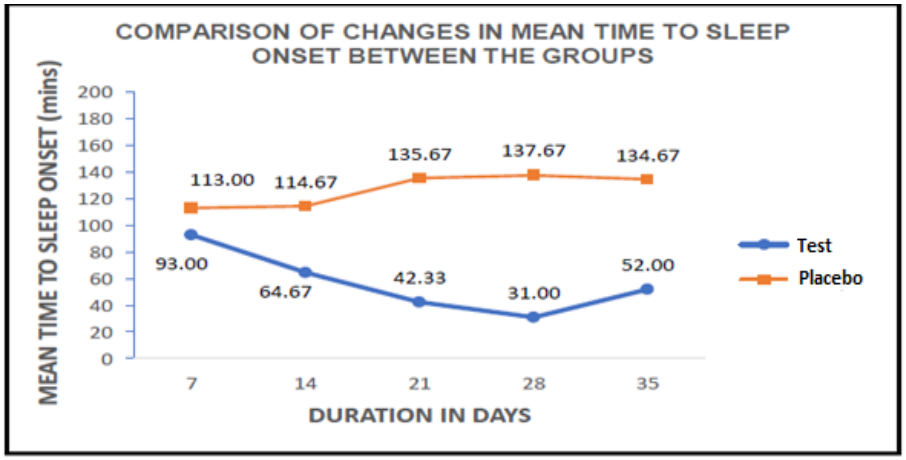
3.2.3 Patient-reported time to sleep onset (as per patient diary) between the groups

In test drug group, the mean patient-reported time to sleep onset on day 7 was 93.00 ± 41.20 mins, which significantly reduced to 31.00 ± 18.86 mins on day 28. The mean patient-reported time to sleep onset increased from day 28 to 52.00 ± 17.05 mins on day 35, but it significantly reduced from day 7. In placebo group, the mean patient-reported time to sleep onset on day 7 was 113.00 ± 37.34 mins, which significantly increased to 137.67 ± 44.85 mins on day 28. The mean patient-reported time to sleep onset reduced from day 28 to 134.67 ± 46.29 mins on day 35, but it significantly increased from day 7. On comparison between the groups, Test drug performed significantly better than placebo on day 14, day 21, day 28 and day 35. The details are presented in Table 5 and Graph 3.

Table 5: Patient-reported time to sleep onset (as per patient diary) between the groups

Duration (Days)	Patient reported mean time to sleep onset (mins) (X±SD)		P value
	Test Drug (N = 30)	Placebo (N = 30)	
07	93.00 ± 41.20	113.00 ± 37.34	0.053 (NS)
14	64.67 ± 33.53	114.67 ± 43.77	*0.001
21	42.33 ± 19.99	135.67 ± 44.23	*0.001
28	31.00 ± 18.86	137.67 ± 44.85	*0.001
35	52.00 ± 17.05	134.67 ± 46.29	*0.001

By Student t Test *Significant NS = Not Significant



Graph 3: Patient-reported time to sleep onset (as per patient diary) between the groups.

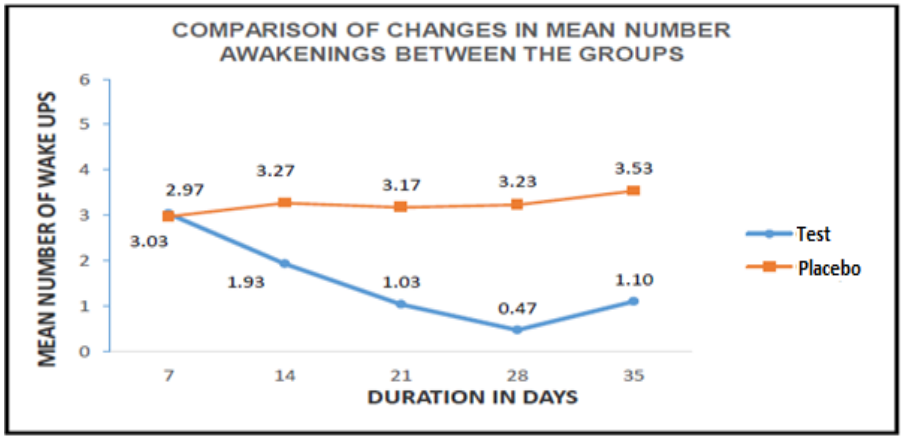
3.2.4 Patient- reported number of awakenings (as per patient diary) between the groups

In test drug group, the mean patient-reported number of awakenings on day 7 was 3.03± 1.43, which significantly reduced to 0.47 ±0.68 on day 28, this increased from day 28 to 1.10 ± 0.66 on day 35, but was significantly lower than day 7. In placebo group, the mean patient-reported number of awakenings on day 7 was 2.97 ± 1.40, which significantly increased to 3.23 ±1.74 on day 28, this increased from day 28 to 3.53 ± 1.83 on day 35, was significantly higher from day 7. After comparison between the groups, Test drug performed significantly better than Placebo on day 14, day 21, day 28 and day 35. The details are presented in Table 6 and Graph 4.

Table 6: Patient-reported number of awakenings (as per patient diary) between the groups.

Duration (Days)	Patient-reported mean number of awakenings (X±SD)		P value
	Test Drug (N = 30)	Placebo (N = 30)	
07	3.03± 1.43	2.97 ± 1.40	0.870 (NS)
14	1.93 ± 1.20	3.27 ± 1.60	*0.001
21	1.03 ± 0.85	3.17 ± 1.56	*0.001
28	0.47 ± 0.68	3.23 ± 1.74	*0.001
35	1.10 ± 0.66	3.53 ± 1.83	*0.001

By Student t Test *Significant NS = Not Significant



Graph 4: Patient-reported number of awakenings (as per patient diary) between the groups.

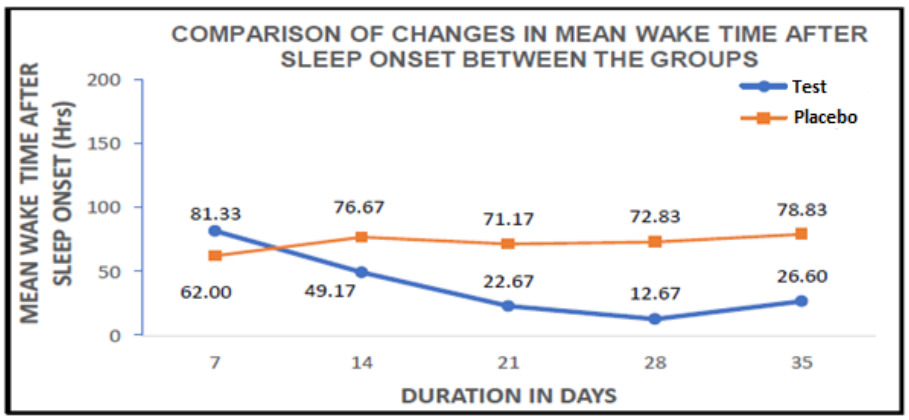
3.2.5 Patient -reported wake time after sleep onset (WASO) between the groups

In test drug group, the mean WASO on day 7 was 81.33 ± 45.01 mins, which significantly reduced to 12.67 ± 20.54 mins on day 28, this increased from day 28 to 26.60 ± 20.64 mins on day 35, but was significantly lower from day 7. In placebo group, the mean WASO on day 7 was 62.00 ± 31.20 mins, which significantly increased to 72.83 ± 36.52 mins on day 28, this increased from day 28 to 78.83 ± 44.52 mins on day 35, was also significantly higher from day 7. On comparison between the groups, Test drug, performed significantly better than Placebo on day 14, day 21, day 28 and day 35. The details are presented in Table 7 and Graph 5.

By Student t Test *Significant NS = Not Significant

Table 7: Patient -reported wake time after sleep onset (WASO) between the groups.

Duration (Days)	Mean WASO (mins) (X±SD)		P value
	Test Drug (N = 30)	Placebo (N = 30)	
07	81.33 ± 45.01	62.00 ± 31.20	0.058 (NS)
14	49.17 ± 35.91	76.67 ± 40.44	*0.007
21	22.67 ± 20.71	71.17 ± 37.68	*0.001
28	12.67 ± 20.54	72.83 ± 36.52	*0.001
35	26.60 ± 20.64	78.83 ± 44.52	*0.001



Graph 5: Patient -reported wake time after sleep onset between the groups.

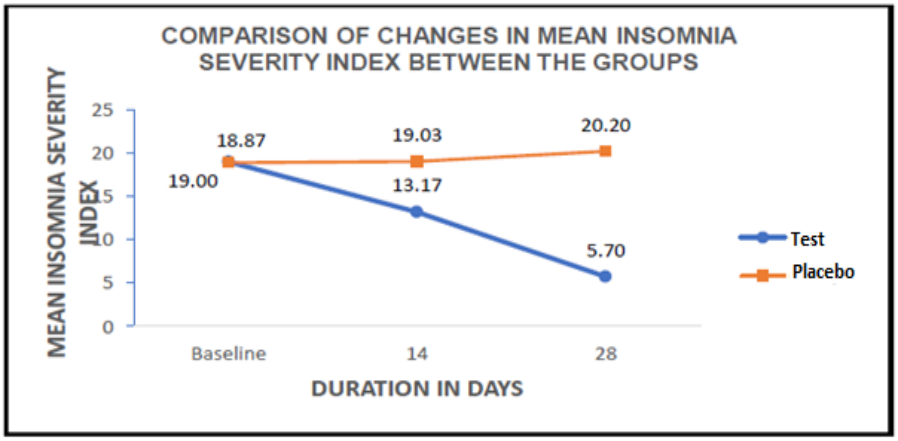
3.2.6 Severity of insomnia assessed using insomnia severity index between the groups

In test drug group, the mean severity of insomnia on baseline visit was 19.00 ± 2.00, which significantly reduced to 13.17 ± 3.23 and 05.70 ± 4.23 on day 14 and 28 respectively. In placebo group, the mean severity of insomnia on baseline visit was 18.87 ± 1.91, which increased significantly to 19.03 ± 2.71 and 20.20 ± 2.72 on day 14 and 28 respectively. When compared between the groups, Test drug performed significantly better than placebo on day 14 and day 28. The details are presented in Table 8 and Graph 6.

Table 8: Severity of insomnia assessed using insomnia severity index between the groups.

Duration (Days)	Mean Insomnia Severity Index (X±SD)		P value
	Test Drug (N = 30)	Placebo (N = 30)	
Baseline	19.00 ± 2.00	18.87 ± 1.91	0.797 (NS)
14	13.17 ± 3.23	19.03 ± 2.71	
28	05.70 ± 4.23	20.20 ± 2.72	
Mean diff (Baseline –14 Days) (P value)	*-05.83 ± 2.91 (0.001)	0.17 ± 1.95 (0.636) NS	*0.001
Mean diff (Baseline –28 Days) (P value)	*-13.30 ± 4.14 (0.001)	*01.33 ± 2.89 (0.017)	*0.001

By Wilcoxon Sign Rank Test *Significant NS = Not Significant By Mann Whitney U Test



Graph 6: Severity of insomnia assessed using insomnia severity index between the groups.

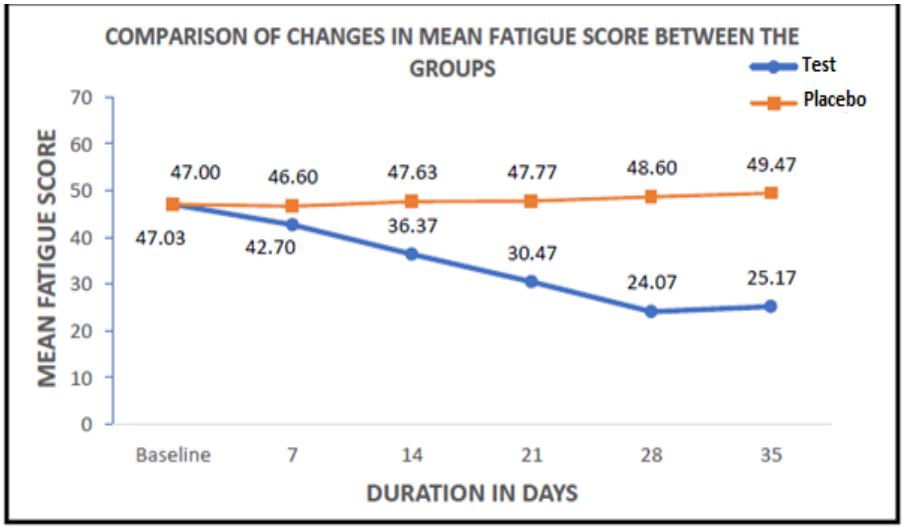
3.2.7 Daytime fatigue assessed using Fatigue Severity Scale (FSS) between the groups

In test drug group, the mean daytime fatigue at baseline visit was 47.03 ± 7.83, which significantly reduced to 24.07 ± 6.34 on day 28, this slightly increased from day 28 to 25.17 ± 7.72 on day 35, but ~~it~~ was significantly lower from baseline visit. In placebo group, the mean daytime fatigue on baseline visit was 47.00 ± 6.34, which increased from baseline visit to 48.60 ± 7.30 on day 28, this increased from day 28 to 49.47 ± 8.04 on day 35, was also higher from baseline visit. When compared between the groups, test drug performed significantly better than placebo on day 7, day 14, day 21, day 28 and day 35. The details are presented in Table 9 and Graph 7.

Table 9: Daytime fatigue assessed using Fatigue Severity Scale (FSS) between the groups

Duration (Days)	Mean Daytime Fatigue (X±SD)		P value
	Test Drug (N = 30)	Placebo (N = 30)	
Baseline	47.03 ± 7.83	**47.00 6.34**	0.987 (NS)
07	42.70 ± 7.44	46.60 ± 6.85	
14	36.37 ± 7.39	47.63 ± 7.71	
21	30.47 ± 5.90	47.77 ± 8.43	
28	24.07 ± 6.34	48.60 ± 7.30	
35	25.17 ± 7.72	49.47 ± 8.04	
Mean diff (Baseline–7 Days) (P value)	*-4.33 ± 3.62 (0.001)	-0.40 ± 2.85 (0.448) NS	*0.001
Mean diff (Baseline –14 Days) (P value)	*-10.67 ± 5.79 (0.001)	0.63 ± 4.11 (0.407) NS	*0.001
Mean diff (Baseline –21 Days) (P value)	*-16.57 ± 6.53 (0.001)	0.77 ± 4.45 (0.350) NS	*0.001
Mean diff (Baseline –28 Days) (P value)	*-22.97 ± 7.17 (0.001)	1.60 ± 4.36 (0.053) NS	*0.001
Mean diff (Baseline –35 Days) (P value)	*-21.87 ± 9.84 (0.001)	*2.47 ± 5.66 (0.023)	*0.001

By ANOVA *Significant NS = Not Significant



Graph 7: Daytime fatigue assessed using Fatigue Severity Scale (FSS) between the groups.

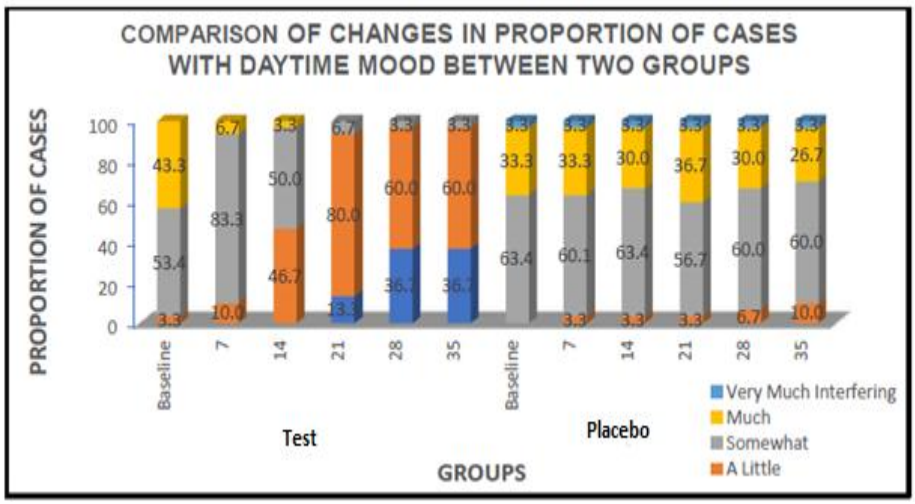
3.2.8 Assessment of daytime mood between the groups

When compared between the groups the change was significantly more among in subjects of test drug group than subjects of placebo group on day 7, 14, 21, 28 and 35. The details are presented in Table 10 and Graph 8.

Table 10: Assessment of daytime mood between the groups.

Daytime mood	Test Drug						Placebo					
	B No %	D7 No %	D14 No %	D21 No %	D28 No %	D35 No %	B No %	D7 No %	D14 No %	D21 No %	D28 No %	D35 No %
	30	30	30	30	30	30	30	30	30	30	30	30
Not at all Interfering,	- (-)	- (-)	- (-)	04 (13.3)	11 (36.7)	11 (36.7)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
A Little	01 (03.3)	03 (10.0)	14 (46.7)	24 (80.0)	18 (60.0)	18 (60.0)	- (-)	01 (03.3)	01 (03.3)	01 (03.3)	02 (06.7)	03 (10.0)
Somewhat	16 (53.4)	25 (83.3)	15 (50.0)	02 (6.7)	01 (3.3)	01 (3.3)	19 (63.4)	18 (60.1)	19 (63.4)	17 (56.7)	18 (60.0)	18 (60.0)
Much	13 (43.3)	*02 (06.7)	*01 (03.3)	*- (-)	*- (-)	*- (-)	10 (33.3)	@10 (33.3)	@09 (30.0)	@11 (36.7)	@09 (30.0)	@08 (26.7)
Very Much Interfering	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	01 (03.3)	@01 (03.3)	@01 (03.3)	@01 (03.3)	@01 (03.3)	@01 (03.3)

By Chi Square Test *P < 0.05, Significant
@Between groups P < 0.05, Significant



Graph 8: Assessment of daytime mood between the groups.

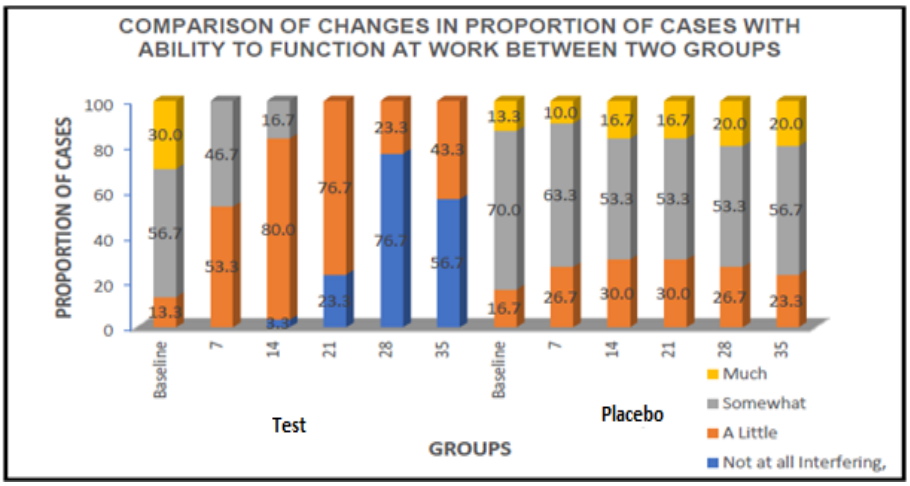
3.2.9 Assessment of ability to function at work between the groups

When compared between the groups the change was significantly more among subjects of test drug group than subjects of placebo group on day 14, 21, 28 and 35. The details are presented in Table 11 and Graph 9.

Table 11: Assessment of ability to function at work between the groups.

Daytime mood	Test Drug						Placebo					
	B No %	D7 No %	D14 No %	D21 No %	D28 No %	D35 No %	B No %	D7 No %	D14 No %	D21 No %	D28 No %	D35 No %
	30	30	30	30	30	30	30	30	30	30	30	30
Not at all Interfering,	- (-)	- (-)	01 (3.3)	07 (23.3)	23 (76.7)	17 (56.7)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
A Little	04 (13.3)	16 (53.3)	24 (80.0)	23 (76.7)	07 (23.3)	13 (43.3)	05 (16.7)	08 (26.7)	09 (30.0)	09 (30.0)	08 (26.7)	07 (23.3)
Somewhat	17 (56.7)	14 (46.7)	*05 (16.7)	*- (-)	*- (-)	*- (-)	21 (70.0)	19 (63.3)	@16 (53.3)	@16 (53.3)	@16 (53.3)	@17 (56.7)
Much	09 (30.0)	- (-)	- (-)	- (-)	- (-)	- (-)	04 (13.3)	03 (10.0)	@05 (16.7)	@05 (16.7)	@06 (20.0)	@06 (20.0)

By Chi Square Test *P < 0.05, Significant
@Between groups P < 0.05, Significant



Graph 9: Assessment of ability to function at work between the groups.

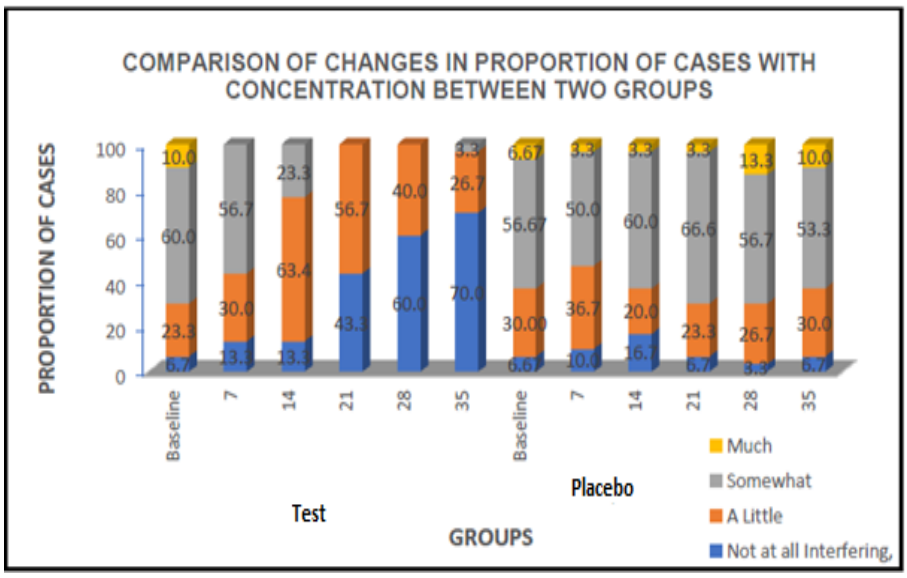
3.2.10 Assessment of concentration between the groups

When compared between the groups the change was significantly better among subjects of test drug group than subjects of placebo group on day 14, 21, 28 and 35. The details are presented in Table 12 and Graph 10.

Table 12: Assessment of concentration between the groups.

Daytime mood	Test Drug						Placebo					
	B No %	D7 No %	D14 No %	D21 No %	D28 No %	D35 No %	B No %	D7 No %	D14 No %	D21 No %	D28 No %	D35 No %
	30	30	30	30	30	30	30	30	30	30	30	30
Not at all Interfering,	02 (6.7)	04 (13.3)	04 (13.3)	13 (43.3)	18 (60.0)	21 (70.0)	02 (6.7)	03 (10.0)	05 (16.7)	02 (6.7)	01 (3.3)	02 (6.7)
A Little	07 (23.3)	09 (30.0)	19 (63.4)	17 (56.7)	12 (40.0)	08 (26.7)	09 (30.0)	11 (36.7)	06 (20.0)	07 (23.3)	08 (26.7)	09 (30.0)
Somewhat	18 (60.0)	17 (56.7)	*07 (23.3)	*- (-)	*- (-)	*01 (3.3)	17 (56.7)	15 (50.0)	@18 (60.0)	@20 (66.6)	@17 (56.7)	@16 (53.3)
Much	03 (10.0)	- (-)	- (-)	- (-)	- (-)	- (-)	02 (6.7)	01 (3.3)	@01 (3.3)	@01 (3.3)	@04 (13.3)	@03 (10.1)

By Chi Square Test *P < 0.05, Significant
@Between groups P < 0.05, Significant



Graph 10: Assessment of concentration between the groups.

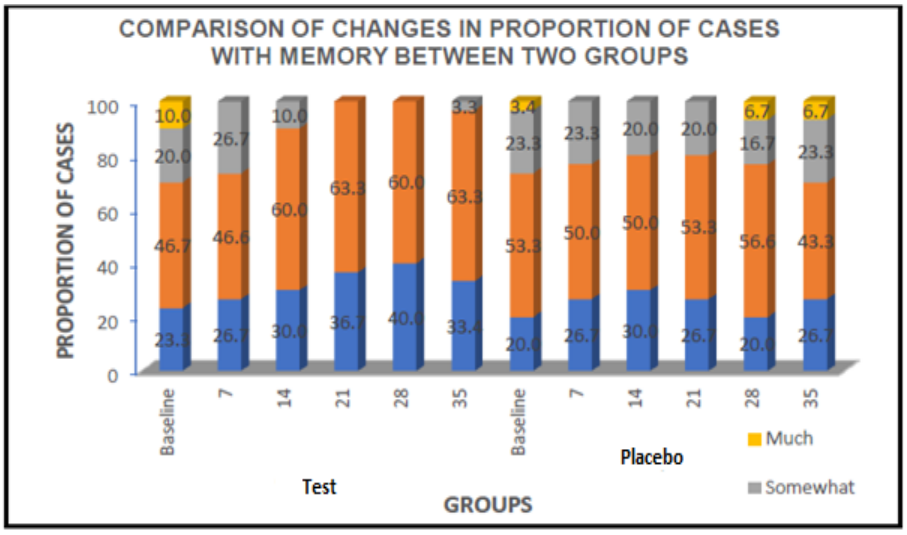
3.2.11 Assessment of memory between the groups

On comparison between the groups the change was significantly better among subjects of test drug group than subjects of placebo group on day 21, 28 and 35. The details are presented in Table 13 and Graph 10.

Table 13: Assessment of memory between the groups.

Daytime mood	Test Drug						Placebo					
	B No %	D7 No %	D14 No %	D21 No %	D28 No %	D35 No %	B No %	D7 No %	D14 No %	D21 No %	D28 No %	D35 No %
	30	30	30	30	30	30	30	30	30	30	30	30
Not at all Interfering,	07 (23.3)	08 (26.7)	09 (30.0)	11 (36.7)	12 (40.0)	10 (33.4)	06 (20.0)	08 (26.7)	09 (30.0)	08 (26.7)	06 (20.0)	08 (26.7)
A Little	14 (46.7)	14 (46.6)	18 (60.0)	19 (63.3)	18 (60.0)	19 (63.3)	16 (53.3)	15 (50.0)	15 (50.0)	16 (53.3)	17 (56.6)	13 (43.3)
Somewhat	06 (20.0)	08 (26.7)	03 (10.0)	*- (-)	*- (-)	*01 (3.3)	07 (23.3)	07 (23.3)	06 (20.0)	@06 (20.0)	@05 (16.7)	@07 (23.3)
Much	03 (10.0)	- (-)	- (-)	- (-)	- (-)	- (-)	01 (3.4)	- (-)	- (-)	@- (-)	@02 (6.7)	@02 (6.7)

By Chi Square Test *P < 0.05, Significant
@Between groups P < 0.05, Significant



Graph 11: Assessment of memory between the groups.

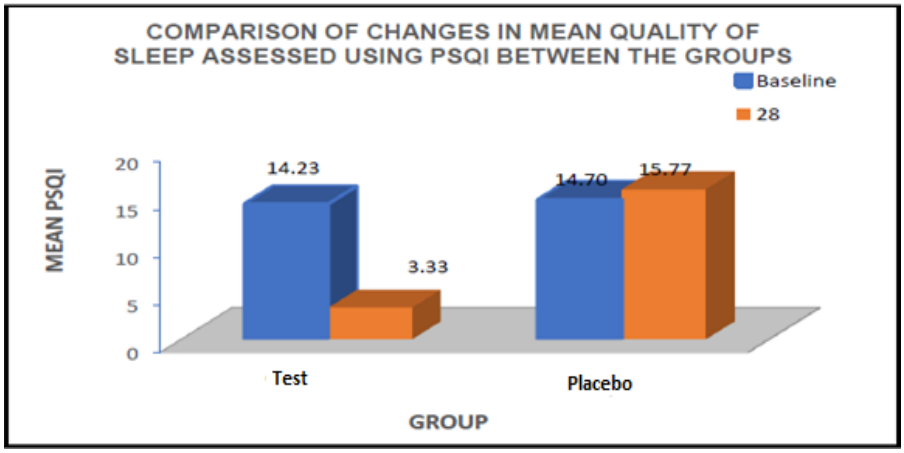
3.2.12 Quality of sleep assessed using Pittsburgh Sleep Quality Index (PSQI) between the groups

In test drug group, the mean quality of sleep assessed using PSQI on baseline visit was 14.23 ± 2.03, which significantly improved to 3.33 ± 2.02 on day 28. In placebo group, the mean quality of sleep assessed using PSQI on baseline visit was 14.70 ± 1.95, which significantly increased to 15.77 ± 1.33 on day 28. When compared between the groups, test drug performed significantly better than placebo. The details are presented in Table 14 and Graph 12.

Table 14: Quality of sleep assessed using Pittsburgh Sleep Quality Index (PSQI) between the groups.

Duration (Days)	Mean PSQI (X ± SD)		P value
	Test Drug (N = 30)	Placebo (N = 30)	
Baseline	14.23 ± 2.03	14.70 ± 1.95	0.364 (NS)
28	3.33 ± 2.02	15.77 ± 1.33	
Mean diff (Baseline –28 Days) (P value)	*-10.90 ± 1.83 (0.001)	*1.07 ± 1.76 (0.002)	*0.001

Wilcoxon Sign Rank Test *Significant NS = Not Significant By Mann Whitney U Test



Graph 12: Quality of sleep assessed using Pittsburgh Sleep Quality Index (PSQI) between the groups.

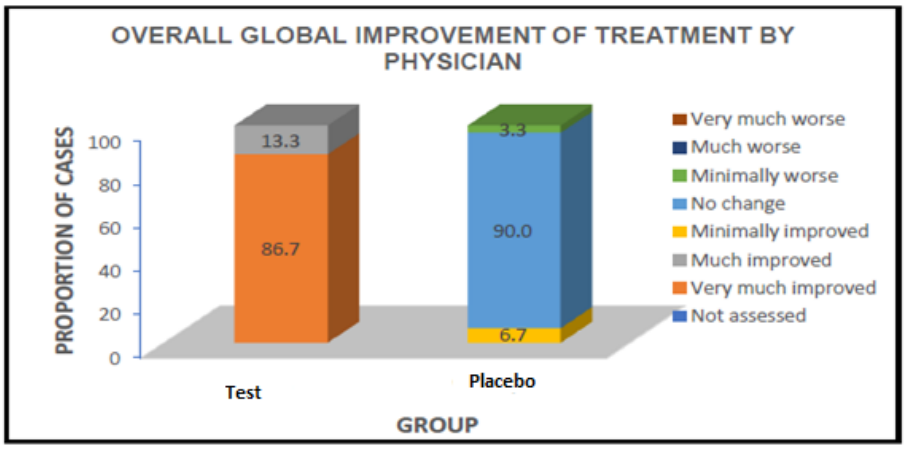
3.2.13 Global assessment for overall improvement by investigator

In test drug group, 26 (86.7%) subjects reported very much overall improvement and 04 (13.3%) subjects reported much overall improvement at the end of the study. In placebo group, 02 (6.7%) subjects reported minimum overall improvement, 27 (90%) subjects reported no change and 1 (3.3%) subject reported minimal worsening in the condition at the end of the study. When compared between the groups, test drug performed significantly better than placebo. The details are presented in Table 15 and Graph 13.

Table 15: Global assessment for overall improvement by investigator.

Assessment	Test Drug (N= 30)		Placebo (N= 30)	
	No.	%	No.	%
Not assessed	-	-	-	-
Very much improved	*26	86.7	-	-
Much improved	*04	13.3	-	-
Minimally improved	-	-	02	06.7
No change	-	-	27	90.0
Minimally worse	-	-	01	03.3
Much worse	-	-	-	-
Very much worse	-	-	-	-

By Chi Square Test *P < 0.05, Significant



Graph 13: Global assessment for overall improvement by investigator.

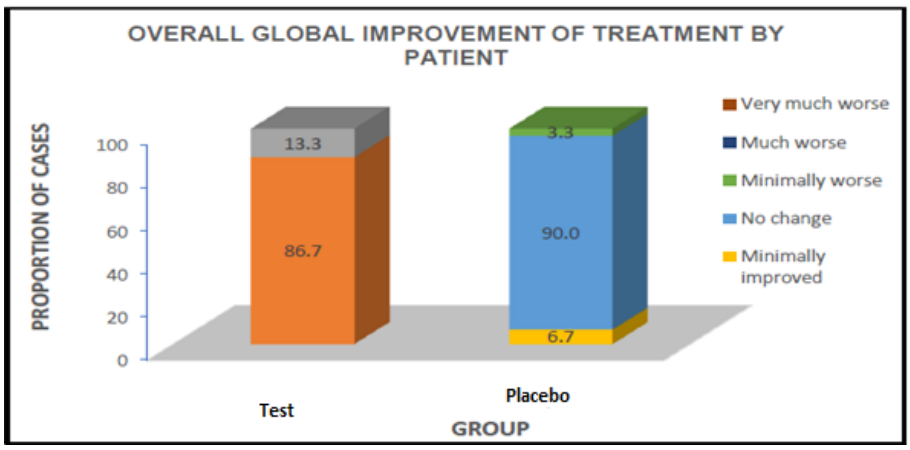
3.2.14 Global assessment for overall improvement by subject

In test drug group, 26 (86.7%) subjects reported very much overall improvement and 04 (13.3%) subjects reported much overall improvement at the end of the study. In placebo group, 02 (6.7%) subjects reported minimal overall improvement, 27 (90%) subjects reported no change and 1 (3.3%) subject reported minimal worsening in the condition at the end of the study. When compared between the groups, test drug performed significantly better than placebo. The details are presented in Table 16 and Graph 14.

Table 16: Global assessment for overall improvement by subject.

Assessment	Test Drug (N= 30)		Placebo (N= 30)	
	No.	%	No.	%
Not assessed	-	-	-	-
Very much improved	*26	86.7	-	-
Much improved	*04	13.3	-	-
Minimally improved	-	-	02	06.7
No change	-	-	27	90.0
Minimally worse	-	-	01	03.3
Much worse	-	-	-	-
Very much worse	-	-	-	-

By Chi Square Test *P < 0.05, Significant



Graph 14: Global assessment for overall improvement by subject.

3.3 Safety Assessment

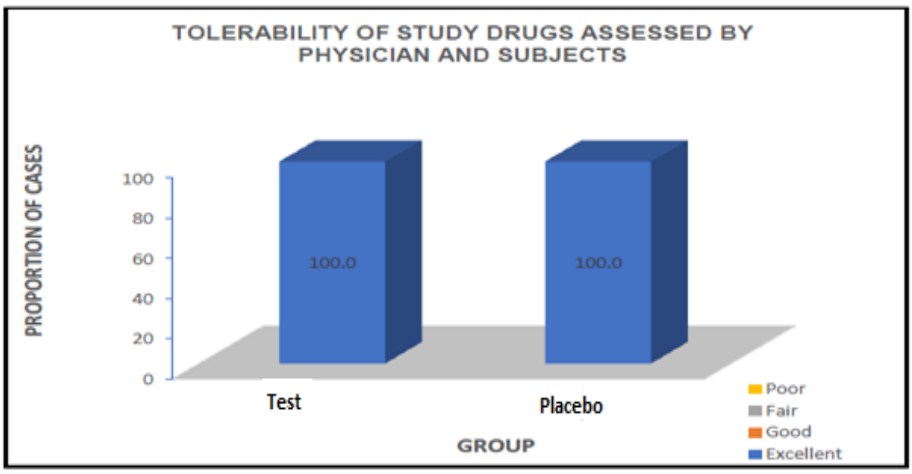
3.3.1 Tolerability of study drugs assessed by physician and subjects

As per physician and subjects, all the subjects (100%) from both the groups reported excellent tolerability to given medication. The details are presented in Table 17 and Graph 15.

Table 17: Tolerability of study drugs assessed by physician and subjects.

Assessment	Test Drug (N= 30)		Placebo (N= 30)	
	No.	%	No.	%
Excellent	30	100.0	30	100.0
Good	-	-	-	-
Fair	-	-	-	-
Poor	-	-	-	-

By Chi Square Test P > 0.05, Not significant



Graph 15: Tolerability of study drugs assessed by physician and subjects.

3.3.2 Profile of adverse events

In test drug group, 9 (30%) subjects reported a total of 15 adverse events during the study period. These adverse events included fever, menstrual pain, acidity, hyperacidity, injury, body ache, dry & irritable eyes, cold, heartburn, back pain and headache. All these adverse events were mild in severity except headache which was moderate in nature. These adverse events were resolved completely after rescue medication was given. Study treatment was not stopped during these adverse events. All these adverse events were not related to the study drug. In placebo group, 11 (36.7%) subjects reported a total of 13 adverse events during the study period. These adverse events included fever, menstrual pain, headache, vomiting, lumbar pain, body ache, mouth ulcer, acidity and leg pain. All these adverse events were mild in severity and resolved completely after rescue medication was given. Study treatment was not stopped during these adverse events. All these adverse events were not related to the study drug. When compared between the groups, the difference was statistically insignificant. The details are presented in Table 18.

Table 18: Profile of adverse events.

Events	Test Drug (N= 30)		Placebo (N= 30)	
	No.	%	No.	%
Fever	03	20	03	23.1
Vomiting	-	-	01	07.7
Menstrual pain	01	06.7	02	15.4
Headache	01	06.7	01	07.7
Hyperacidity	02	13.3	-	-
Injury	01	06.7	-	-
Lumbar pain	-	-	01	07.7
Body ache	01	06.7	01	07.7
Dry & irritable eyes	01	06.7	-	-
Cold	02	13.3	-	-
Heartburn	01	06.7	-	-
Mouth ulcer	-	-	02	15.4
Back pain	-	-	-	-
Acidity	01	06.7	01	07.7
Leg pain	01	06.7	01	07.7
No of Patients	**09**	**30.0**	**11**	**36.7**
No of events	**15**		**13**	

By Chi Square Test P > 0.05, Not significant

3.4 Lab investigation

All the laboratory parameters were within normal limits at baseline visit in both the groups. After completion of the treatment, no significant change in any of the laboratory parameters was observed in both the groups. If compared between the groups, the difference was statistically insignificant.

4. DISCUSSION

Patients receiving test drug showed significant benefit in total sleep time and mean sleep efficiency, the primary endpoints of the study. Significant improvement in the mean total sleep time and mean sleep efficiency was observed from 7 days onwards till day 28 in test drug group. Stoppage of treatment after 28 days, although led to slight decline in mean total sleep time and mean sleep efficiency on day 35 in patient receiving test drug, these were significantly increased from baseline values suggesting there is no significant relapse after stoppage of treatment for 7 days. From these results it is evident that test drug is significantly superior to placebo in terms of improving total sleep time and sleep efficiency in subjects suffering from primary insomnia and significant rebound insomnia is not observed.

The mean time to sleep onset, mean number of total awakenings and mean WASO significantly reduced on day 28 in test drug group. The mean time to sleep onset, mean number of total awakenings and mean WASO significantly increased on day 28 in placebo. Even after stoppage of treatment for 7 days, the mean time to sleep onset, the mean number of total awakenings and WASO significantly reduced from baseline to day 35 in subjects of test drug group, suggesting there is no significant relapse in these symptoms.

On day 28, the mean insomnia severity index significantly reduced from baseline visit by 30.7% in test drug group, whereas it insignificantly increased from baseline visit by 0.8% on day 28 in placebo group. At the beginning of the study, subjects of test drug group were in moderate clinical insomnia category as per insomnia severity index. After 28 days of treatment almost all the subjects were in no clinically significant insomnia category as per insomnia severity index. Subjects from placebo group remained in moderate clinical insomnia category during the entire study period. This suggests that test drug is superior to placebo in terms of reducing severity of insomnia in subjects suffering from primary insomnia.

The mean global PSQI score reduced significantly from 14.23 on baseline visit to 3.33 on day 28 in test drug group; however the score increased from 14.70 on baseline visit to 15.77 on day 28 in subjects from Placebo. This suggests that the quality of sleep was significantly improved in test drug group than placebo group at the end of treatment.

Significant improvement was observed in symptoms such as fatigue, daytime mood, ability to function at work, concentration and memory on day 28 in test drug group, however no significant improvement was observed in these symptoms on day 28 in subjects from placebo group. Overall improvement in primary insomnia was assessed by subjects and physician at the end of the study. It was observed that 86.7% subjects from test drug group reported very much improvement, whereas 90% subjects from placebo group reported no change in primary insomnia at the end of the study.

The superiority of test drug in improving primary insomnia over placebo could be attributed to the synergistic effect of the ingredients present in the formulation. Tagara one of the important ingredient present in test drug is used as an anti-anxiety and sleep inducing drug in the treatment of insomnia. It was observed in the studies that valerenic acid and valepotriates present in Tagar are responsible for sedative action. A possible mechanism by which these compounds may cause effectiveness is by increasing the quantity of GABA, an inhibitory neurotransmitter in the central nervous system that plays a role in the etiology of insomnia. There are also some evidences that suggest that Tagara may cause GABA to be released from brain nerve endings and then block GABA from reuptake into nerve cells. In addition, Tagara inhibits an enzyme that destroys GABA.[22-25] Tagara contains isovaltrate, which may exhibit stimulatory effects on the central nervous system by blockade of tonically activated adenosine A1 receptors in the brain and thus useful for sleep-inducing effect.[26]

Ashwagandha is reported to possess sleep promoting effect by linking GABAergic modulation, which is significantly antagonized.[27,28] *Ashwagandha* contains withanolide glycosides and withaferin A these help reduce stress-induced insomnia.[29-31] Brahmi contains bacosides that are responsible for improving vital neurotransmitters action which happen in memorization and evidence process and may be supportive in depression.[32-36] The sedative action of Nutmegh is due to the presence of compounds like Myristicin and Safrole. It has been observed in many studies that Nutmegh may increase serotonergic activity in brain, resulting in anti-depressant effect.[37-40] Jatamansi prevents enzyme induced breakdown of GABA in the brain resulting in sedation[41-45] Shankhapushpi helps in inducing a feeling of calm and peace, encourages good sleep and carries relief in anxiety and mental fatigue. It brings a substantial decrease in anxiety level. Shankhapushpi exhibits significant antidepressant like effect by interaction with the adrenergic, dopaminergic, and serotonergic systems.[46-48]

Fever, menstrual pain, acidity, hyperacidity, body ache, dry & irritable eyes, cold, heartburn, back pain, leg pain, mouth ulcer, vomiting and headache were adverse events observed in both the groups. These adverse events were

resolved completely after rescue medication was given. These episodes did not require interruption of the study drugs or procedure, hence were not related to the study drugs or procedure. The difference in adverse event profile of both the groups was statistically insignificant.

Excellent tolerability of both the drugs was reported by subjects and physician at the end of the study. No significant post treatment change in any of the lab investigations was observed in both the groups. Also no significant post treatment change in vitals such as pulse rate, blood pressure, body temperature and respiratory rate was observed in both the groups, suggesting both the drugs were safe in subjects suffering from primary insomnia. Thus Test drug is safe and effective in subjects suffering from primary insomnia.

5. CONCLUSION

Test drug is safe in subjects suffering from primary insomnia. Test drug was significantly effective in improving total sleep time and sleep efficiency and in reducing time to sleep onset, total number of awakenings, wake time after sleep onset and severity of insomnia in subjects suffering from primary insomnia. The significant effect of test drug in primary insomnia can be seen after 7 days of treatment. Symptoms associated with primary insomnia such as fatigue, problems in daytime mood, ability to function at work, concentration and memory were also significantly improved. Significantly improvement in quality of sleep in subjects suffering from insomnia was also noticed. Stoppage of treatment for seven days did not result in significant rebound of insomnia. Thus test drug is safe and effective medicine for the treatment of primary insomnia without significant rebound insomnia effect usually ascribed to sleep medications.

6. Conflicts of interest

There are no conflicts of interest.

7. Funding

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