Research Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2024.54.008564

Efficacy of a Multilayer Nutraceutical Compound in Subjects with Poor Sleep Quality: A Double Blind, Randomized Placebo-Controlled Study

Alessandra Castelnuovo[1,2], Alessandro Oldani[1], Francesca Marta Casoni[1], Samantha Mombelli[1], Stefania Murzilli[3]*, Arianna Vanelli[2] and Sara Marelli[1,2]

[1]*Alessandra Castelnuovo, Alessandro Oldani, Francesca Marta Casoni, Samantha Mombelli, and Sara Marelli IRCCS Ospedale San Raffaele Turro - UO Neurologia - Centro di Medicina del Sonno, via Stamira D'Ancona, 20, 20127 Milan (MI), Italy*

[2]*Alessandra Castelnuovo and Sara Marelli "Università Vita-Salute San Raffaele", via Olgettina, 58, 20132 Milano (MI), Italy*

[3]*Research and Development, Biofarma group SRL, part of Biofarma Group, Italy*

***Corresponding author:** Stefania Murzilli, Research and Development, Biofarma group SRL, part of Biofarma Group, Italy

ARTICLE INFO

Received: January 02, 2024

Published: January 11, 2024

Citation: Alessandra Castelnuovo, Alessandro Oldani, Francesca Marta Casoni, Samantha Mombelli, Stefania Murzilli, Arianna Vanelli and Sara Marelli. Efficacy of a Multilayer Nutraceutical Compound in Subjects with Poor Sleep Quality: A Double Blind, Randomized Placebo-Controlled Study. Biomed J Sci & Tech Res 54(3)-2024. BJSTR. MS.ID.008564.

ABSTRACT

Introduction: Sleep is essential for maintaining good health as poor sleep quality can increase the risk of physical and mental diseases. The prevalence of poor sleep quality reaches 94% in adults. Many individuals who complain of sleep-related difficulties present hyperarousal, a state of cognitive and physiological activation. Sedatives and hypnotics can provoke misuse or tolerance, while recent studies report the benefit of nutraceuticals in treating this situational condition without these consequences.

Materials and Methods: This double blind, randomized, placebo-controlled study evaluated the effects of a multilayer nutraceutical compound containing saffron, Eucommia Ulmoides and Magnolia Officinalis taken for 27 days. Sixty-seven participants aged 18 to 63 years, with disturbed and poor sleep quality, were randomly assigned to the active and placebo groups. Participants completed self-reported questionnaires (PSQI, PSAS and ISI) before and after the treatment period and a sleep diary every morning.

Results: No statistically significant differences were observed when comparing the nutraceutical compound to placebo. However, statistically significant changes were observed in both arms after a 27-days active treatment. After the nutraceutical compound administration, the PSQI global score reached 5.57±3 from 8.11±2.47, the cognitive and somatic domains of PSAS decrease of -4.22 (-5.36 ÷ -3.07) and -1.59 (-2.34 ÷ -0.83) points, respectively, the ISI score was reduced from 12.7±4.98 to 9.38±5.79.

Discussion: The nutraceutical compound was effective in improving sleep quality, but measurements may be affected by the subjects' expectations (i.e., placebo effect) and the results should be carefully interpreted. Further larger investigation is suggested.

Keywords: Poor Sleep Quality; Saffron; Eucommia Ulmoides; Magnolia Officinalis

Abbreviations: PSQI: Pittsburgh Sleep Quality Index; PSAS: Pre-sleep Arousal Scale; ISI: Insomnia Severity Index; TST: Total Sleep Time; SE: Sleep efficiency

Introduction

Sleep is an essential factor to maintain good health [1]. Literature found a prevalence of 6-94% of poor sleep quality in adults [2]. Recently, sleep quality is defined as one's satisfaction of the sleep experience, integrating aspects of sleep initiation, sleep maintenance, sleep quantity, and refreshment upon awakening [3]. The National Sleep Foundation released the key indicators of good sleep quality, including increase in sleeping time while in bed (at least 85% of the total time), falling asleep in 30 min or less, waking up no more than once per night and being awake for 20 min or less after initially falling asleep [4]. Poor sleep quality is associated with significantly decreased work performance and impaired daytime function [5] and can affect social and occupational behaviours [6]. Subjects who complain of disturbed and poor sleep often present high levels of arousal [7]. Hyperarousal is a state of cognitive and physiological activation

that can be constantly present: this state can result in a long sleep latency, in relation to the difficulty in "turning off" the centres of wakefulness [8]. It is well known that individuals with difficulty in falling asleep show increased sympathetic autonomic activity [8-10]. Several studies have reported a close relationship between cortical and autonomic arousal, emphasizing that elevated sympathetic activity is associated with increased cortical arousal [10-13].

Considering the hyperarousal model of Riemann and colleagues [14], according to which hyperactivity of the arousal system and hypoactivity of the sleep system commutatively cause insomnia, patients with difficulty in falling asleep due to high levels of arousal might benefit from compounds that can reduce cognitive and physiological activation during the evening hours. Drugs are often taken by patients with insomnia or poor sleep quality, when it is not possible or useful to conduct a cognitive behavioural therapy for insomnia (CBT-I; the first-line treatment for insomnia). While effective, sedative and hypnotics have the potential for abuse, cross-reactivity with other medications, and side effects including memory loss, abnormal thoughts, behavioural changes, and headaches [15,16]. Alternatively, food supplements, like valerian [17,18] and passionflower [19,20], can be used. Valerian was found to almost double the chance of sleeping better when compared with placebo in a recent systematic review with 16 clinical studies [17]. Compared to placebo, passionflower as tea yielded short-term sleep benefits for healthy adults with mild fluctuations in sleep quality [19] and increased total sleep time and sleep efficiency [20]. Remarkable positive effects of other nutraceuticals had been also mentioned in the treatment of sleep disorders [21]. The purpose of this work was to assess the efficacy of the multilayer nutraceutical compound, composed of saffron, Eucommia Ulmoides and Magnolia Officinalis, in adult subjects with disturbed, poor sleep quality at night, by measuring the cognitive and somatic arousal which is associated with pre-sleep arousal activity [6,22].

Materials and Methods

Study Design

This is a monocentric, doubleblind, randomized trial designed to assess preliminary efficacy of the multilayer nutraceutical compound, composed of saffron, Eucommia Ulmoides and Magnolia Officinalis, in adult subjects with disturbed, poor-quality sleep at night. Subjects were randomly assigned to one of the following treatment arms:

- Multilayer nutraceutical compound 1200 mg tablets
- Placebo tablets (identical to the nutraceutical product in shape, size, colour and taste).

Self-administered questionnaires that can assess both cognitive and somatic pre-sleep arousal and sleep quality were used. The first objective of the study was to evaluate the change from baseline to end-of-study visit in the mean Pittsburgh Sleep Quality Index (PSQI) score (primary efficacy variable) in a direct comparison between active and placebo treatments. In addition, the changes of the Pre-sleep Arousal Scale (PSAS) and Insomnia Severity Index (ISI) scores from baseline were evaluated vs placebo at the end-of-study visit. All subjects filled in the PSQI, PSAS and ISI questionnaires over the study. During the treatment phase, subjects were provided with a sleep diary, in which they documented on a daily basis details about the quality of sleep and the conditions upon awakening. Data on demographic, physical examination, previous and concomitant medications and treatment compliance were collected. A physical examination was performed at baseline (Day 1) and Visit 3 (Day 28). Possible ad-verse events were recorded through the study. This study was approved by the Ethics Committee of "IRCCS Ospedale San Raffaele". In-formed consent was obtained from all participants prior to enrolment.

Participants

Participants were included if they met the following inclusion criteria:

i. Men and women aged between 18 and 65;

ii. PSQI score >5;

iii. PSAS score >15 on at least one of the two domains;

iv. Ability to understand and sign informed consent.

Potential participants were excluded if they

(i) Were unable to read or understand and correctly complete the study procedures;

(ii) Had circadian rhythm sleep-wake disorders or Restless Legs Syndrome;

(iii) Had major respiratory disorders (respiratory failure, pneumopathy, pneumothorax, Chronic Obstructive Pulmonary Disease) and Obstructive Sleep Apnoea Syndrome;

(iv) Were affected by nocturnal epilepsy, Rapid Eye Movement and Not Rapid Eye Movement parasomnias;

(v) Had neurological, psychiatric, and/or cardiac disorders (clinically significant);

(vi) Had any form of dementia or cognitive decline;

(vii) Were being treated with benzodiazepines, z-drugs (zopi-clone, eszopiclone, zaleplon and zolpidem), antidepressants and neuroleptics;

(viii) Were allergic to pollen, saffron, magnolia, magnesium, Eucommia Ulmoides and other components of the investigational product;

(ix) Were pregnant or lactating women;

(x) Were potentially fertile women unwilling to use barrier contraceptive methods during the study.

Participants were recruited from October 2019 to October 2021, with a period of shutdown, due to the pandemic. During a pre-screening visit, 12 possible participants were not invited to baseline (Day 1) as they did not respect the inclusion criteria (e.g., were treated with

benzodiazepines, had clinically significant insomnia or other psychiatric disorders). At baseline (Day 1), 67 participants, who signed the informed consent, were enrolled and randomly assigned to either the active (n=37) or placebo (n=30) group. The participant flow diagram is shown in Figure 1.

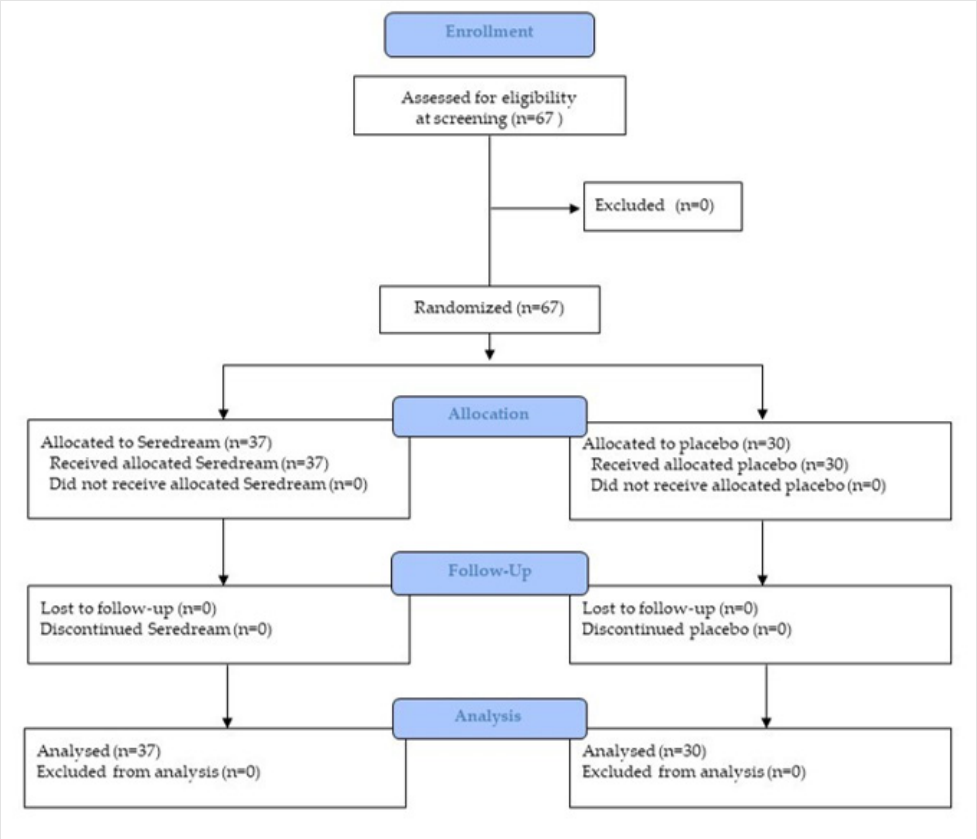

Figure 1: Participants flow diagram.

Self-Administered Questionnaires

Sleep quality, pre-sleep arousal and severity of insomnia were assessed by the questionnaires described below:

- Pittsburgh Sleep Quality Index (PSQI; Primary Efficacy Variable): PSQI is one of the most widely used questionnaires in the assessment of sleep quality. It was developed to provide a reliable, valid and standardized measure of sleep quality through a survey covering the last month. The use of this instrument is justified by the good psychometric properties that characterize it and by the presence of cut-off scores. The cut-off of the scale is represented by a score greater than 5, considered as pathological [23-25].

- Pre-Sleep Arousal Scale (PSAS; Secondary Efficacy Variable): This scale consists of 16 items (8 for the somatic domain and 8 for the cognitive domain) that aim to assess pre-sleep arousal. Responses range from 1 (not at all) to 5 (very much), with a range of scores between 8 and 40, for each of the 2 domains [26, 27].

- Insomnia Severity Index (ISI; Secondary Efficacy Variable): The scale was validated in 2011 by Morin's group [28] and the cut-off was set at 10. The scale was translated in Italian and validated in 2016 by Castronovo and their colleagues [29]. This instrument, consisting of 5 questions, is used to determine the severity and impact of insomnia on the patient's life. The ISI is a scale consisting of 7 items, through which the subject assesses sleep difficulty in terms of severity (divided into three different areas: "difficulty falling asleep," "difficulty staying asleep," and "early awakening problem"), degree of interference with daytime efficiency, evidence of such impairment to others, level of discomfort, and overall satisfaction with one's sleep. Responses are distributed on a Likert scale from 0 to 4. The total score ranges from 0 to 28 and is assessed as follows: 0-7 no clinically significant insomnia; 8-14 insomnia below the critical threshold; 15-21 clinical insomnia (medium severity); 22-28 clinical insomnia (severe). Subjects were provided with a sleep diary (secondary efficacy variable) to document on a daily basis details about the quality of sleep and

the conditions upon awakening. The sleep diary was completed by the subject every morning after awakening for 28 days. The variables taken into consideration for the statistical analysis are:

1) Bedtime
2) Sleep Latency
3) Wake after Sleep Onset
4) Time in Bed
5) Total Sleep Time
6) Sleep Efficiency
7) Number of Awakenings
8) Sleep Quality (during the previous night)
9) Tiredness and Fatigue (during the previous day).

A Visual Analogic Scale (VAS; secondary efficacy variable) for product satisfaction was also included. It consists of a segment (100 mm) on which the subject must indicate the perceived level of satisfaction between the two extremes 0= "Not at all" and 100= "Very much".

Investigational Product

Each tablet of the multilayer nutraceutical product contains the following natural compounds: saffron (15 mg), Eucommia Ulmoides (200 mg), and Magnolia Officinalis (200 mg). The first layer, composed of saffron slows down the heartbeats of the subject allowing a gradual falling asleep. This reduction in blood pressure occurs thanks to the action of safranal, one of the active ingredients of saffron, as found in several studies [30,31]. Magnesium, which contributes to the reduction of fatigue and tiredness, has also been included in the same layer. In addition, magnesium is considered anti-stress as it protects the heart and blood circulation from the effects of stress [32]. The second layer is composed of Eucommia Ulmoides. This plant is considered a sedative capable of increasing sleep time and quality [33]. Additionally, Eucommia reduces blood pressure through inhibition of the beta-adrenergic system, thus allowing for a reduction in fatigue. The third layer includes Magnolia Officinalis, which is used as a remedy to promote relaxation. This plant contains phenolic compounds that interact with the GABA A receptor and with the cortisol hormone. A study by Kuribara and colleagues showed that Magnolia Officinalis has muscle relaxant and anxiolytic activity. In addition, it has a regularizing effect on the stomach and intestinal mucosa [34]. The three layers are released at different times to allow for optimal action of all components. The first layer has a fast release, to allow the relaxation of the individual and the consequent gradual falling asleep.

The second layer has a delayed release to allow continuous sleep during all its phases, avoiding intra-sleep awakenings thanks to the sedative effect. The third layer has an intermediate release to act immediately after the relaxing action of saffron, therefore acting on the falling asleep phase. The product (i.e., nutraceutical compound or placebo) was taken in the evening, 30-60 minutes before bedtime. The duration of treatment was from Day 1 to Day 27 (unused product was collected on Day 28). Treatment compliance was checked at Visit 2 (Day 14) and Visit 3 (Day 28).

Statistical Methods

Assuming a standard deviation estimate of the changes from baseline to the final visit in PSQI scores of 2.44 in the active and 2.94 in the placebo treatment arm and an estimated mean difference between active and placebo of 1.87 in the final change vs. baseline of the aforementioned PSQI score, we calculated that a sample size of 67 subjects should provide 80% power to detect a difference as statistically significant with a significance level (alpha) of 0.05 and using a t-test for two independent samples. Estimates of standard deviations and mean difference between treatments were extrapolated from the literature [35]. Calculations were performed using PASS 14 software. The analysis of the primary efficacy variable was performed using a linear model (ANCOVA) with treatment and baseline PSQI score as covariates and the difference in PSQI score from baseline as the dependent variable. The analysis of all secondary efficacy parameters was performed using an ANCOVA model with treatment and baseline measurements as covariates and change from baseline as the de-pendent variable. We used an ANCOVA model as we were mainly interested in treatments comparison at the last follow-up visit (time by time comparison) and not to the comparisons of time-course pro-files of treatments. Results are reported as mean differences between treatments with 95% confidence intervals and two-tailed probability values.

Results

Baseline Characteristics

There were no significant differences between the two groups at baseline. Background characteristics of the 67 enrolled participants are shown in Table 1. Thirty patients (45%) were female:15 (41%) in the treatment group and 15 (50%) in the placebo group, with a mean age of 36.76±13.93 years in the active group (N=37) and 35.5±12.2 years in the placebo group (N=30).

Table 1: Summary of demographic, and anamnestic data.

Variable	Active	Placebo
Age (years)		
Mean ± SD (N)	36.76±13.93 (37)	35.5±12.2 (30)
Median (min - max)	29 (18 - 63)	31 (20 - 59)
Gender (female)	40.5 (15/37)	50 (15/30)
Ethnic (Caucasian)	100 (37/37)	100 (30/30)
Weight (kg)		
Mean ± SD (N)	72.11±10.6 (37)	70.32±12.09 (30)
Median (min - max)	71 (52 - 92)	72 (45 - 90)
Height (cm)		
Mean ± SD (N)	174.24±8.44 (37)	173.3±8.09 (30)
Median (min - max)	175 (155 - 191)	175 (160 - 185)
BMI		
Mean ± SD (N)	23.71±2.73 (37)	23.34±3.28 (30)
Median (min - max)	23.51 (18.9 - 29.41)	22.87 (17.36 - 32.03)

Note: Categorical variables are summarized as percentage and absolute frequency versus the n° of subjects in the ITT population [%(n/N)]; continuous variables are summarized as mean, SD, n° of non-missing observations in the ITT population, median, minimum and maximum.

At baseline, the subjects in both the active and the placebo arms suffered from no insomnia at all (51.4% in the active arm and 56.7% in the placebo arm) or only from not clinically significant insomnia (48.6% in the active arm and 43.3% in the placebo arm).

Pittsburgh Sleep Quality Index (PSQI)

After the treatment period, a slight improvement in sleep quality was observed in both the active and the placebo group with no statistically significant between-group difference in the mean PSQI global score, as reported in Table 2. Of note, the PSQI – global score significantly improved from baseline (Day 1) to Visit 3 (Day 28), after the nutraceutical compound administration (8.11±2.47 vs 5.57±3; p <.0001). In particular, in the active arm statistically significant differences of some components were detected, as reported in Table 3. After a 27-days active treatment, we observed a delay in wake-up time (6:48 ± 0:46 vs 7:07 ± 0:53; p=0.026), a decrease in sleep latency (34.19 ± 27.34 min vs 23.43 ± 16.65 min; p=0.005), an increase in total sleep time (355.68 ± 55.00 min vs 396.08 ± 54.60 min; p<0.005), and an improvement in sleep efficiency (78.22 ± 10.75 vs 85.53 ± 10.84; p<0.005).

Table 2: Pittsburgh Sleep Quality Index (PSQI) Global Score - Inferential Statistics.

Outcome	Active	Placebo	Difference	p-Value
PSQI - Global Score	-2.52 (-3.20 ÷ -1.84)	-2.69 (-3.44 ÷ -1.94)	-0.17 (-1.18 ÷ 0.84)	0.7349

Note: Results are reported as LS means change from baseline with associated two-tailed 95% CI and p-values. ANCOVA model is based on the change from baseline to endpoint with fixed effects for treatment and baseline value as a covariate. All p-values, LS means, and confidence intervals are calculated from the ANCOVA model. No missing data imputation (LOCF) was performed since no missing data occurred in the ITT population of these efficacy variables

Pre-Sleep Arousal Scale (PSAS) and Insomnia Severity Index (ISI)

No statistically significant pre- and post-treatment differences were observed between the two treatment groups, however, subjects treated with the nutraceutical compound showed a reduction in pre-sleep arousal. The mean value of the cognitive domain decreased (23.89±5.52 vs 19.43±5.62; p<.0001), as well as the mean value of the somatic domain (14.43±4.86 vs 12.59±4.16; p=0.0009) from baseline to Visit 3 (Day 28). The descriptive statistics of the PSAS domains are reported in Supplemental Table 1. This finding indicates that subjects were less activated both cognitively and somatically at the time of falling asleep after the nutraceutical compound administration. However, even in the placebo group, the PSAS score of the cognitive and somatic domains fell at Visit 3 (Day 28) and no statistically significant differences were found by comparing the nutraceutical compound with placebo (Table 4). Overall, severity of insomnia (ISI score) was reduced in the active group from baseline to Vis-it 3 (Day 28) (12.7±4.98 vs 9.38±5.79; p<.0001). A reduction in the ISI score was observed over time even in the placebo group, with no statistically significant differences between the treatment groups (Table 5).

Table 3: PSQI domains and Global Score of the active group - Descriptive Statistics.

PSQI - Sleep quality			
Baseline		**Visit 3**	
Mean ± SD (N)	1.97±0.55 (37)	Mean ± SD (N)	1.38±0.64 (37)
Median (Min - Max)	2 (1 - 3)	Median (Min - Max)	1 (0 - 3)
PSQI - Sleep latency			
Baseline		**Visit 3**	
Mean ± SD (N)	1.62±0.86 (37)	Mean ± SD (N)	1.27±0.96 (37)
Median (Min - Max)	2 (0 - 3)	Median (Min - Max)	1 (0 - 3)
PSQI - Sleep duration			
Baseline		**Visit 3**	
Mean ± SD (N)	1.32±0.88 (37)	Mean ± SD (N)	0.7±0.74 (37)
Median (Min - Max)	1 (0 - 3)	Median (Min - Max)	1 (0 - 3)
PSQI - Sleep efficiency			
Baseline		**Visit 3**	
Mean ± SD (N)	1.11±1.02 (37)	Mean ± SD (N)	0.46±0.77 (37)
Median (Min - Max)	1 (0 - 3)	Median (Min - Max)	0 (0 - 3)
PSQI - Sleep disturbances			
Baseline		**Visit 3**	
Mean ± SD (N)	1±0.24 (37)	Mean ± SD (N)	0.97±0.29 (37)
Median (Min - Max)	1 (0 - 2)	Median (Min - Max)	1 (0 - 2)
PSQI - Daytime dysfunction			
Baseline		**Visit 3**	
Mean ± SD (N)	1.08±0.83 (37)	Mean ± SD (N)	0.78±0.82 (37)
Median (Min - Max)	1 (0 - 3)	Median (Min - Max)	1 (0 - 3)
PSQI - Global Score			
Baseline		**Visit 3**	
Mean ± SD (N)	8.11±2.47 (37)	Mean ± SD (N)	5.57±3 (37)
Median (Min - Max)	7 (4 - 15)	Median (Min - Max)	5 (2 - 16)

Table 4: Pre-Sleep Arousal Scale (PSAS) Domains - Inferential Statistics.

Outcome	Active	Placebo	Difference	p-value
PSAS – Cognitive	-4.22 (-5.36 ÷ -3.07)	-4.33 (-5.61 ÷ -3.06)	-0.12 (-1.85 ÷ 1.62)	0.8923
PSAS – Somatic	-1.59 (-2.34 ÷ -0.83)	-2.31 (-3.15 ÷ -1.47)	-0.72 (-1.86 ÷ 0.41)	0.2073

Note: Results are reported as LS means change from baseline with associated two-tailed 95% CI and p-values. ANCOVA model is based on the change from baseline to endpoint with fixed effects for treatment and baseline value as a covariate. All p-values, LS means, and confidence intervals are calculated from the ANCOVA model. No missing data imputation (LOCF) was performed since no missing data occurred in the ITT population of these efficacy variables.

Table 5: Insomnia Severity Index (ISI) - Descriptive and Inferential Statistics.

ISI Score	Active	Placebo
Baseline		
Mean ± SD (N)	12.7±4.98 (37)	10.53±2.66 (30)
Median (Min - Max)	11 (6 - 25)	11 (6 - 16)
Visit 3		
Mean ± SD (N)	9.38±5.79 (37)	6.8±3.55 (30)
Median (Min - Max)	7 (2 - 27)	7 (0 - 15)
LS Means (95% CL)	-3.24 (-4.31 ÷ -2.18)	-3.83 (-5.02 ÷ -2.65)
LS Difference (95% CL) - p-value	-0.59 (-2.21 ÷ 1.03) – P = 0.4704	

Note: Results are reported as LS means change from baseline with associated two-tailed 95% CI and p-values. ANCOVA model is based on the change from baseline to endpoint with fixed effects for treatment and baseline value as a covariate. All p-values, LS means, and confidence intervals are calculated from the ANCOVA model. No missing data imputation (LOCF) was performed since no missing data occurred in the ITT population of these efficacy variables.

Sleep Diary

The data obtained from the sleep diaries were in line with what was observed from the PSQI questionnaire. No statistically significant pre- and post-treatment differences were observed between the two treatment groups. Considering this, we decided to evaluate pre- and post-treatment in the two groups individually. We found statistically significant differences in almost all sleep diary parameters in both groups after 27 days of treatment. We decided to focused only on active group because we were not interested in placebo effect. (Table 6). In particular, decreased sleep latency (-21.22±79.77; p=0.0059), increased sleep efficiency (2.91±7.85; p= 0.0026), reduced number of nocturnal awakening (-0.31±0.72; p= 0.0025) and overall level of tiredness and fatigue during previous day (-0.57±1.22; p= 0.0053) were observed at Visit 3 (Day 28). No other statistically significant difference was found between the study treatments when comparing the results of the sleep diary, as shown in Supplemental Table 2.

Table 6: Sleep Diary Outcomes - Active arm.

Sleep Diary Endpoint	Baseline (Average)	Follow-up (Visit 3 Average)	Difference (Visit 3) – (Baseline)	p-value †
Bedtime (change from midnight)				
Mean ±SD (N)	-0.54±0.74	-0.65±0.7	-0.10±0.63	0.1906
Sleep latency (min.)				
Mean ±SD (N)	48.20±68.85	26.98±36.97	-21.22±79.77	0.0059
Wake after sleep onset (min.)				
Mean ±SD (N)	27.23±23.42	19.67±35.82	-7.56±33.81	0.0018
Time in bed (hours)				
Mean ±SD (N)	7.81±0.75	7.98±0.73	0.17±0.71 (35)	0.1488
Total sleep time (hours)				
Mean ±SD (N)	6.82±0.98	7.17±0.90	0.35±0.88 (36)	0.0121
Sleep efficiency (%)				
Mean ±SD (N)	87.10±7.89	90.01±7.98	2.91±7.85 (35)	0.0026
Number of nocturnal awakening				
Mean ±SD (N)	1.43±1.07	1.12±1.21	-0.31±0.72	0.0025
Sleep quality during the last night				
Mean ±SD (N)	5.88±1.58	6.33±1.65	0.45±1.32	0.0722
Overall level of tiredness and fatigue during previous day				
Mean ±SD (N)	5.20±1.74	4.63±2.05	-0.57±1.22	0.0053

Note: Data are summarized as mean, SD, n° of non-missing observations in the ITT Population. † P-values are computes using the nonparametric Wilcoxon Rank Sum Test.

Discussion

The overall aim of this study was to evaluate the preliminary efficacy of the multilayer nutraceutical compound in adult subjects with disturbed, poor-quality sleep at night through the assessment of self-assessment questionnaires. Pre- and post-treatment analyses showed no significant differences between the active and the placebo groups in the PSQI questionnaire, nor in the PSAS in both domains. Furthermore, no significant differences between the groups were observed in the ISI and VAS questionnaires nor in the sleep diaries parameters. The primary objective of the study was not demonstrated. Analyses on the active group showed a significant reduction in all PSQI components except for the sleep disturbance, the time spent in bed and time going to bed. There was a decrease in asleep latency of 11 minutes, an increase in Total Sleep Time (TST) as subjects tended to sleep approximately 40 minutes longer after the nutraceutical administration. Sleep efficiency (SE%) also presented statistically significant differences, as its score increased from 87 to 90. A reduction in the ISI score and in the pre-asleep arousal (both domains) was observed, indicating that subjects had less difficulty sleeping and were less cognitively and somatically stimulated at the time of falling asleep, after active treatment administration. The product was generally quite satisfying for the subjects.

Finally, in line with the results obtained from the compilation of the PSQI, significant differences were also found in the sleep diaries in almost all the variables investigated except for the total time spent in bed and the actual time at which the subjects went to bed, probably because these cannot be controlled by the effect of the nutraceutical product, as they represent typically subjective components. Similarly in a previous randomized double-blind controlled clinical trial, which concluded that six weeks of saffron supplementation improved sleep quality, sleep latency, sleep duration, and led to a PSQI global score equal to 6.46 [36]. However, a reduction in the PSQI global score was even observed in the placebo group and the trial did not achieve its primary objective of detecting a statistically significant difference between the nutraceutical compound and placebo in the mean change of the PSQI global score (p=0.7349) from baseline (Day 1) to Visit 3 (Day 28). Results of the present study are comparable to those of other pharmacological studies, as reported in literature.

The meta-analysis of Buscemi et al., reported that benzodiazepines provoke a reduction of 10 minutes in sleep latency [37], while melatonin provokes a variable reduction in sleep latency depending on the dosage [37,38]. Combining the results of the meta-analysis by Buscemi, et al. [37] and the one by Ferracioli-Oda, et al. [38], melatonin caused a mean reduction of sleep latency (active versus placebo) equal to 5.5 minutes.

Other compounds, such as ramelteon and suvorexant, were associated to a similar reduction in sleep latency of 9 minutes when compared to placebo [39,40]. In the study of Kuriyama, et al. [39], ramelteon was associated with improvement in persistent sleep, total sleep time, and sleep efficiency; there-fore, short-term use of ramelteon was associated with improvement in some sleep parameters in patients with insomnia, but its clinical impact resulted small. The analysis of Kuriyama et al., 2016, including four randomized trials involving 3076 patients [40], suggested that suvorexant was associated with significant improvements in time to sleep onset, total sleep time, and quality of sleep at 1 and 3 months. On the other hand, several adverse events, such as sleepiness, fatigue, and abnormal dreams were detected. The efficacy results need to be considered taking into account that difficulties in falling asleep and difficulties in maintaining sleep may be influenced by external factors and the measurements should be carefully interpreted as they may be affected by the expectations of the subjects [41]. The use of placebos may become unconditioned stimuli for 'down regulation' or sleep itself [42]. The use of the placebo could represent a form of conditioning that, augmented by social suggestions or therapeutic rituals, lead the study population to manifest benefits despite not having taken an active product [43-49]. Several studies in literature have also investigated the placebo effect related to hypnotic drugs.

A study conducted in 2012, showed that placebo and hypnotic drugs led to similar improvements, with only a 22-minute difference in sleep latency and a 7-minute difference in sleep latency assessed by polysomnography [49]. These improvements were similar to those of this study where a nutraceutical product provoked a decrease in sleep latency by 11 minutes. Other studies compared the effect that different hypnotic drugs and nutraceutical products have on the sleep latency over several weeks [37,38,50]. It has been seen that nutraceuticals, with different treatment durations and doses, had greater variability on sleep latency and actual improvements compared to hypnotic drugs. In conclusion, despite the lack of statistically significant differences between the two arms, the multilayer nutraceutical product succeeds in this study, by improving subjective sleep quality, and confirming that further research on the nutraceutical product under investigation, may provide more stable and consistent results. A couple of limitations of this study need to be considered. First, at baseline (Day 1) the sub-jects in the placebo arm showed a reduced PSQI (7.93±2.29) if compared with similar studies on supplements for sleep disturbances [35]. Therefore, a significant difference among the active and the placebo groups was less detectable. Second, the sample size was limited and this study might be underpowered to detect a meaningful difference between treatments. A larger sample size is needed for further studies.

Acknowledgments

We thank the whole staff of OPIS s.r.l. involved in this study for the technical support.

Research Funding

This research was funded by Biofarma group s.r.l.

Author Contributions

Conceptualization, Alessandra Castelnuovo, Stefania Murzilli, Arianna Vanelli and Sara Marelli; Data curation, Francesca Casoni; Formal analysis, Francesca Casoni; Investigation, Alessandra Castelnuovo and Sara Marelli; Methodology, Samantha Mombelli; Project administration, Stefania Murzilli; Resources, Alessandro Oldani; Supervision, Sara Marelli; Validation, Alessandra Castelnuovo and Alessandro Oldani; Visualization, Samantha Mombelli; Writing – original draft, Alessandra Castelnuovo; Writing – review & editing, all authors.

Competing Interests

The funder participated to the setup of the study design and to the redaction of the publication. However, the funder had no role in data collection, analyses, and interpretation of the data.

Informed Consent

Informed consent was obtained from all subjects involved in the study.

Ethical Approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of "IRCCS Ospedale San Raffaele" (protocol code Seredream, v.02 – approved on 27 May 2019).

References

1. Léger D, Poursain B, Neubauer D, Uchiyama M (2008) An international survey of sleeping problems in the general population. Current Medical Research and Opinion 24(1): 307-317.
2. Simonelli G, Marshall NS, Grillakis A, Miller CB, Hoyos CM, et al. (2018) Sleep health epidemiology in low and middle-income countries: A systematic review and meta-analysis of the prevalence of poor sleep quality and sleep duration. Sleep Health 4(3): 239-250.
3. (2013) Encyclopedia of Behavioral Medicine. In: Gellman MD, Turner JR (Eds.)., New York, NY: Springer New York.
4. Ohayon M, Wickwire EM, Hirshkowitz M, Albert SM, Avidan A, et al. (2017) Nation-al Sleep Foundation's sleep quality recommendations: first report. Sleep Health 3(1): 6-19.
5. Wang WL, Chen KH, Pan YC, Yang SN, Chan YY, et al. (2020) The effect of yoga on sleep quality and insomnia in women with sleep problems: a systematic review and meta-analysis. BMC Psychiatry 20(1): 195.
6. Dement WC, Miles LE, Carskadon MA (1982) "White Paper" on Sleep and Aging. Journal of the American Geriatrics Society 30(1): 25-50.
7. Halász P, Terzano M, Parrino L, Bódizs R (2004) The nature of arousal in sleep. J Sleep Res 13(1): 1-23.
8. Barnard K, Broman-Fulks J, Michael K, Webb R, Zawilinski L, et al. (2011) The effects of physiological arousal on cognitive and psychomotor performance among individuals with high and low anxiety sensitivity. Anxiety, stress and coping 24(2): 201-216.
9. Monroe LJ (1967) Psychological and physiological differences between good and poor sleepers. J Abnorm Psychol 72(3): 255-264.
10. Adam K, Tomeny M, Oswald I (1986) Physiological and psychological differences between good and poor sleepers. J Psychiatr Res 20(4): 301-316.
11. Nelson J, Harvey AG (2003) An exploration of pre-sleep cognitive activity in insomnia: imagery and verbal thought. Br J Clin Psychol 42(Pt 3): 271-288.
12. Sforza E, Juony C, Ibanez V (2002) Time-dependent variation in cerebral and autonomic activity during periodic leg movements in sleep: implications for arousal mechanisms. Clin Neurophysiol 113(6): 883-891.
13. Abbott SBG, Coates MB, Stornetta RL, Guyenet PG (2013) Optogenetic stimulation of c1 and retrotrapezoid nucleus neurons causes sleep state-dependent cardiorespiratory stimulation and arousal in rats. Hypertension 61(4): 835-841.
14. Riemann D, Spiegelhalder K, Feige B, Voderholzer U, Berger M, et al. (2010) The hy-perarousal model of insomnia: a review of the concept and its evidence. Sleep Med Rev 14(1): 19-31.
15. Holbrook AM, Crowther R, Lotter A, Cheng C, King D, et al. (2000) Meta-analysis of benzodiaze-pine use in the treatment of insomnia. CMAJ 162(2): 225-233.
16. Buscemi N, Vandermeer B, Friesen C, Bialy L, Tubman M, et al. (2007) The Efficacy and Safety of Drug Treatments for Chronic Insomnia in Adults: A Meta-analysis of RCTs. J Gen Intern Med 22(9): 1335-1350.
17. Bent S, Padula A, Moore D, Patterson M, Mehling W, et al. (2006) Valerian for Sleep: A Systematic Review and Meta-Analysis. The American Journal of Medicine 119(12): 1005-1012.
18. Zare Elmi H khaton, Gholami M, Saki M, Ebrahimzadeh F (2021) Efficacy of Valerian Extract on Sleep Quality after Coronary Artery bypass Graft Surgery: A Triple-Blind Randomized Controlled Trial. Chin J Integr Med 27(1): 7-15.
19. Ngan A, Conduit R (2011) A Double-blind, Placebo-controlled Investigation of the Effects of *Passiflora incarnata* (Passionflower) Herbal Tea on Subjective Sleep Quality: effect of passionflower on subjective sleep quality. Phytother Res 25(8): 1153-1159.
20. Lee J, Jung HY, Lee SI, Choi JH, Kim SG, et al. (2020) Effects of Passiflora incarnata Linnaeus on polysomnographic sleep parameters in subjects with insomnia disorder: a double-blind randomized placebo-controlled study. International Clinical Psychopharmacology 35(1): 29-35.
21. Ali R, Tariq S, Kareem O, Faizana Fayaz, Tahira Aziz, et al. (2021) Nutraceuticals for Sleep Disorders. Comb Chem High Throughput Screen 24(10): 1583-1592.
22. Bruni O, Ferini Strambi L, Giacomoni E, Pellegrino P (2021) Herbal Remedies and Their Possible Effect on the GABAergic System and Sleep. Nutrients 13(2): 530.
23. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ (1989) "The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research." Psychiatry Res 28(2): 193-213.
24. Chesson A, Hartse K, Anderson WM, Davila D, Johnson S, et al. (2000) Practice parameters for the evaluation of chronic insomnia. An American Academy of Sleep Medi-cine report. Standards of Practice Committee of the American Academy of Sleep Medi-cine. Sleep 23(2): 237-241.
25. Sateia MJ, Doghramji K, Hauri PJ, Morin CM (2000) Evaluation of chronic insomnia. An American Academy of Sleep Medicinereview. Sleep 23(2): 243-308.
26. Nicassio PM, Mendlowitz DR, Fussell JJ, Petras L (1985) The phenomenology of the pre-sleep state: the development of the pre-sleep arousal scale. Behav Res Ther 23(3): 263-271.

27. Devoto A, Violani C (2009) Curare l'insonnia senza farmaci - Metodi di valutazione e intervento cognitivo – comportamentale. Roma: In: Carrocci Faber (Edt.).,.
28. Morin CM, Belleville G, Bélanger L, Ivers H (2011) The Insomnia Severity Index: psychometric indicators to detect insomnia cases and evaluate treatment response. Sleep 34(5): 601-608.
29. Castronovo V, Galbiati A, Marelli S, Brombin C, Cugnata F, et al. (2016) Validation study of the Italian version of the Insomnia Severity Index (ISI). Neurol Sci 37(9): 1517-1524.
30. Hosseinzadeh H, Noraei NB (2009) Anxiolytic and hypnotic effect of Crocus sativus aqueous extract and its constituents, crocin and safranal, in mice. Phytother Res 23(6): 768-774.
31. Imenshahidi M, Razavi BM, Faal A, Gholampoor A, Mousavi SM, et al. (2015) The Effect of Chronic Administration of Safranal on Systolic Blood Pressure in Rats. Iran J Pharm Res 14(2): 585-590.
32. Cao Y, Zhen S, Taylor AW, Appleton S, Atlantis E (2018) Magnesium Intake and Sleep Disorder Symptoms: Findings from the Jiangsu Nutrition Study of Chinese Adults at Five-Year Follow-Up. Nutrients 10(10): E1354.
33. Li X, Zhu W, Yang L, Fei D, Fan J, et al. (2013) Evaluation of the sedative and hypnotic effects of Eucommiol in Eucommia. Natural Product Research 27(18): 1657-1659.
34. Kuribara H, Kishi E, Hattori N, Okada M, Maruyama Y, et al. (2000) The Anxiolytic Effect of Two Oriental Herbal Drugs in Japan Attributed to Honokiol from Magnolia Bark. Journal of Pharmacy and Pharmacology 52(11): 1425-1429.
35. Adib Hajbaghery M, Mousavi SN (2017) The effects of chamomile extract on sleep quality among elderly people: A clinical trial. Complementary Therapies in Medicine 35: 109-114.
36. Pachikian BD, Copine S, Suchareau M, Deldicque L (2021) Effects of Saffron Extract on Sleep Quality: A Randomized Double-Blind Controlled Clinical Trial. Nutrients 13(5): 1473.
37. Buscemi N, Vandermeer B, Hooton N, Pandya R, Tjosvold L, et al. (2005) The Efficacy and Safety of Exogenous Melatonin for Primary Sleep Disorders. J Gen Intern Med 20(12): 1151-1158.
38. Ferracioli Oda E, Qawasmi A, Bloch MH (2013) Meta-analysis: melatonin for the treatment of primary sleep disorders. PLoS One 8(5): e63773.
39. Kuriyama A (2014) Ramelteon for the treatment of insomnia in adults: a systematic review and meta-analysis. Sleep Medicine 15(4): 385-392.
40. Kuriyama A, Tabata H (2017) Suvorexant for the treatment of primary insomnia: A systematic review and meta-analysis. Sleep Medicine Reviews 35: 1-7.
41. Finniss DG (2018) Placebo Effects: Historical and Modern Evaluation. In: International Review of Neurobiology. Elsevier, p. 1-27.
42. Perlis ML, McCall WV, Jungquist CR, Pigeon WR, Matteson SE, et al. (2005) Placebo effects in primary insomnia. Sleep Medicine Reviews 9(5): 381-389.
43. Dodd S, Dean OM, Vian J, Berk M (2017) A Review of the Theoretical and Biological Under-standing of the Nocebo and Placebo Phenomena. Clin Ther 39(3): 469-476.
44. Brietzke C, Cesario JCS, Hettinga FJ, Flavio OP (2022) The reward for placebos: mechanisms underpinning placebo-induced effects on motor performance. Eur J Appl Physiol 122: 2321-2329.
45. Winkler A, Rief W (2015) Effect of Placebo Conditions on Polysomnographic Parameters in Primary Insomnia: A Meta-Analysis. Sleep 38(6): 925-931.
46. McCall WV, D Agostino R, Dunn A (2003) A meta-analysis of sleep changes associated with placebo in hypnotic clinical trials. Sleep Medicine 4(1): 57-62.
47. Bélanger L, Vallières A, Ivers H, Moreau V, Lavigne G, et al. (2007) Meta-analysis of sleep changes in control groups of insomnia treatment trials. J Sleep Res 16(1): 77-84.
48. Pan Y, Meister R, Löwe B, Winkelmann A, Kaptchuk TJ, et al. (2019) non-concealed Placebo treatment for menopausal hot flushes: Study protocol of a randomized-controlled trial. Trials 20(1): 508.
49. Huedo Medina TB, Kirsch I, Middlemass J, Klonizakis M, Siriwardena AN, et al. (2012) Effective-ness of non-benzodiazepine hypnotics in treatment of adult insomnia: meta-analysis of data submitted to the Food and Drug Administration. BMJ 345: e8343.
50. Luthringer R, Muzet M, Zisapel N, Staner L (2009) The effect of prolonged-release melatonin on sleep measures and psychomotor performance in elderly patients with insomnia. Int Clin Psychopharmacol 24(5): 239-249.

ISSN: 2574-1241

DOI: 10.26717/BJSTR.2024.54.008564

Stefania Murzilli. Biomed J Sci & Tech Res