

**EFFECT OF HOLIXER TM (OCIMUM TENUIFLORUM EXTRACT) ON SLEEP
MICROSTRUCTURE: A RANDOMIZED DOUBLE-BLIND PLACEBO
CONTROLLED TRIAL**

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ABSTRACT

Background and aims: *Ocimum tenuiflorum*, or holy basil, is well known in Ayurveda for its ability to manage physical and mental health problems. Holixer™, an *Ocimum tenuiflorum* extract, was investigated for its effects on sleep quality, stability, and continuity by examining the microstructure of sleep in healthy stressed individuals. To our knowledge, this is the first study to evaluate the effects of herbal extracts on sleep microstructure.

Experimental procedure: In this 8-week randomized double-blind placebo-controlled study, 70 healthy stressed adults received either Holixer™ (250 mg/day) or placebo. Sleep microstructure (Cyclic Alternating Pattern/CAP rate and CAP subtypes A1, A2, and A3 rates) was assessed as the primary outcome measure using nocturnal polysomnography. The secondary outcomes measured using actigraphy were sleep-onset latency, sleep efficiency, and wakefulness after sleep onset. The Perceived Stress Scale (PSS), Insomnia Severity Index (ISI), and Pittsburgh Sleep Quality Index (PSQI) were used to measure the participants' sleep quality and stress.

Results: Holixer™ significantly increased CAP A1 rates ($p=0.007$) and reduced sleep onset latency ($p=0.02$) and insomnia severity scores ($p=0.005$) compared to the placebo group at week 8. Sleep efficiency ($p=0.07$) and wakefulness after sleep onset ($p=0.08$) showed a trend towards significance when compared with placebo. Holixer™ was well tolerated with no reports of major adverse effects.

Conclusion: The findings of this trial suggest that an 8-week supplementation with Holixer™ improves sleep quality, as evidenced by the microstructural changes observed through polysomnography and participants' experiences measured using validated sleep

questionnaires. (CTRI reg.no: CTRI/2023/10/059316, Registered on: 31/10/2023)

<https://ctri.nic.in/Clinicaltrials/pmaindet2.php?EncHid=OTQzMzY=&Enc=&userName=>

KEYWORDS

Ocimum tenuiflorum, Holy Basil, insomnia, sleep, microstructure, CAP A1, sleep onset latency

1. INTRODUCTION

Sleep disturbances are a well-documented consequence of psychological stress. Stressful life events, chronic anxiety, and heightened emotional arousal have been shown to impair sleep initiation and maintenance, often leading to conditions such as insomnia, fragmented sleep, and reduced overall sleep quality. Conversely, inadequate or poor quality sleep can amplify physiological stress responses in the body, creating a self-perpetuating cycle that negatively affects mental and physical health [1]. Understanding this interplay is critical for developing effective interventions aimed at improving sleep health and mitigating broader health consequences associated with chronic stress. Many studies have reported evidence highlighting the complex bidirectional relationship between stress and sleep quality over the past 50 years [2]. From these reports, it is clear that chronic or perceived stress negatively affects sleep quality [3]. Sleep quality is a crucial indicator of overall health and well-being, and is determined by both the macro- and micro-architecture of sleep. The macro architecture consists of Rapid Eye Movement/REM and Non-REM as major components of sleep, whereas the micro architecture consists of sleep spindles, K complexes, slow-wave activity, and cyclic alternating pattern (CAP). CAP is a periodic EEG pattern characterized by alternating phases of high and low brain activity during non-REM sleep, typically recurring every 20-40 seconds. CAP consists of Phase A (activation) and Phase B (deactivation), where Phase A subdivided into subtypes A1, A2, and A3, each reflecting varying degrees of cortical arousal. An increased CAP rate is considered a marker of sleep instability and has been associated with a range of pathological conditions including insomnia, obstructive sleep apnea, fibromyalgia, and mood disorders. Unlike macro-architectural parameters, CAP and its

phases provide a more sensitive index of sleep fragmentation and arousal burden, offering valuable insight into the neural regulation of sleep and its disruption in disease states [4].

Ocimum tenuiflorum Linn. (Synonym: *Ocimum sanctum*), generally known as Tulsi or Holy Basil, is a perennial herb native to South Asia and belongs to the family Lamiaceae. *O. tenuifolium* has been used in Ayurveda for its adaptogenic/antistress activity. [5, 6]. As an adaptogen, Tulsi helps harmonize stress responses by modulating the hypothalamic–pituitary–adrenal (HPA) axis and reducing cortisol levels, as demonstrated in both preclinical and human trials. In an 8-week, randomized, placebo-controlled study using a standardized extract (Holixer™), stressed adults exhibited a 40% improvement in sleep quality and lowered hair cortisol and acute stress-induced salivary cortisol levels [7]. These findings are corroborated by broader reviews highlighting Tulsi’s role in reducing anxiety, enhancing resilience to stress, and supporting neurochemical pathways linked to sleep and cortisol homeostasis [8]. Taken together, these results underscore the potential of Tulsi as a natural therapeutic agent for mitigating stress-induced sleep disturbances through its multimodal adaptogenic and neuroendocrine actions. The bioactive constituents of Holy Basil, such as apigenin and rosmarinic acid, have been associated with neuroprotective and anxiolytic effects, suggesting a role in stress-induced sleep disorder management. Besides duration, depth and continuity, sleep restorative properties depend on the capacity of the brain to create periods of sustained stable sleep where the subjects are assessed with Cyclic alternating pattern (CAP) which is the EEG marker of unstable sleep [9] [4]. The CAP rate and stages A1, A2, and A3 were significantly correlated with subjective appreciation of sleep quality [10].

Based on this evidence, we hypothesized that Holixer™ supplementation would reduce CAP rates in individuals experiencing moderate levels of perceived stress (Perceived

Stress Scale [PSS] score > 14), as CAP activity is a sensitive objective measure of sleep instability. In addition to CAP metrics, the total sleep time, sleep efficiency, REM duration, REM latency, sleep onset latency, and wakefulness after sleep onset—alongside participants' experience of sleep quality and stress, using validated questionnaires such as the Restorative Sleep Questionnaire (RSQ-W), Insomnia Severity Index (ISI), Pittsburgh Sleep Quality Index (PSQI), and Perceived Stress Scale (PSS) were assessed.

2. MATERIALS AND METHODS

2.1. Study Design and setting

This double-blind, parallel-group, randomized, placebo-controlled trial was conducted at the International Institute of Sleep Sciences (IISS), Maharashtra, India, between the time period September 2023 and February 2025. The manuscript was prepared according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines to ensure transparent and complete reporting of the clinical trial. All participants provided informed consent and the trial protocol was approved by the Independent Ethics Committee at International Institute of Sleep Sciences (Approval number #NRTULS0901-IISS001). This study was prospectively registered with the Clinical Trials Registry-India (CTRI reg.no: CTRI/2023/10/059316, registered on: 31/10/2023).

The study was conducted in three sequential phases: screening, pre-treatment, and treatment (Figure.1).

Screening Phase

Potential participants were invited to the clinical site and provided detailed information about the study and its requirements. Only literate volunteers who could read and understand English were considered eligible to participate. Individuals who provided voluntary written informed consent underwent a screening assessment. These included the Perceived Stress Scale (PSS), STOP-BANG questionnaire for intrinsic severe sleep-related disorders, and Hospital Anxiety and Depression Scale (HADS). Participants who fulfilled the inclusion and exclusion criteria were enrolled and randomized in a double-blind manner to receive either Holixer™ or placebo. All participants were followed up on an outpatient basis, according to the prespecified schedule of visits.

Pre-Treatment Phase

Eligible and consenting individuals were randomized in an allocation-concealed manner into either the Holixer™ or placebo groups. Participants were provided with an actigraphic device (Actiwatch) for seven consecutive days prior to the initiation of the intervention. The capsules were dispensed following collection of the Actiwatch. All 70 participants underwent two consecutive nights of polysomnography (PSG) at baseline (day -1 and day 0) and again on day 56. Assessment scales were administered at the screening visit on Days 28 and 56.

Treatment Phase

Participants attended the clinic on screening day (Day-7), sleep adaptation day, (Day-1), baseline (Day0), day 28, day 49, and day 56. Scheduled telephonic follow-up was done on day7, day14, day 21, 35, and 42.

2.2. Concomitant Medications

Participants were instructed to avoid allopathic or herbal medications during the study period. Concomitant treatment for unrelated conditions was permitted if deemed necessary by the investigator provided that the medications did not interact with the study intervention.

2.3. Randomization and Blinding

The intervention was administered in a double-blind manner, wherein both the participant and investigator were blinded. Randomization was performed using block randomization of varying lengths completed by a statistician not directly involved in participant recruitment, and allocation concealment was ensured using opaque sealed envelopes.

Both participants and researchers remained unaware of the allocation, and strict measures prevented communication or contamination between blinded and unblinded teams. A placebo was selected as the comparator, given its suitability for evaluating CAP outcomes, allowing assessment of clinical relevance without the confounding effects of an active comparator. As the study population consisted of individuals with stress-related sleep disturbances rather than a clinical disease requiring treatment, non-treatment was not expected to result in the deterioration of their condition.

2.4. Inclusion & Exclusion Criteria

Participants were eligible for enrolment if they were between 18 and 65 years of age, had a Perceived Stress Scale (PSS) score greater than 14, and had not used any hypnotic medications or dietary supplements within the preceding three months. All participants were required to read and communicate in English and to provide written informed consent. Women of childbearing potential were required to agree to the use of adequate contraception to prevent pregnancy for the duration of the study. Participants were also instructed to abstain from caffeine and alcohol consumption for at least 12 h prior to undergoing polysomnography.

Participants were excluded if they had a history of ischemic heart disease, valvular heart disease, pacemaker use, or severe respiratory or seizure disorders. Individuals with untreated severe depression, defined as a Hospital Anxiety and Depression Scale (HADS) score ≥ 15 for either anxiety or depression, were not eligible. Participants with severe intrinsic sleep disorders, including severe sleep apnea (STOP BANG score > 5), moderate-to-severe restless legs syndrome, or narcolepsy, were excluded. Women who were

pregnant or nursing or who had a positive pregnancy test at screening or within 48 h prior to the study intervention were not eligible.

The use of disallowed medications, including anxiolytics, central nervous system–active drugs, hypnotics, narcotic analgesics, anti-inflammatory agents, antidepressants, beta blockers, anticonvulsants, St. John’s Wort, sedating H1 antihistamines, kava kava, systemic steroids, Ginkgo biloba, respiratory stimulants, over-the-counter and prescription stimulants, decongestants, diet aids, antipsychotics, melatonin, or any other drugs or supplements known to affect the sleep–wake cycle. Participants with a history of drug addiction, drug abuse, or alcohol abuse (defined as >2 alcoholic drinks per day) within the past 12 months were also excluded. In addition, patients with hepatic impairment (SGOT or SGPT >3× the upper limit of normal) or renal impairment (serum creatinine ≥ 2.0 mg/dL) were excluded. Those with any other severe systemic illness or who were in the opinion of the investigator and were unlikely to comply with the study procedures were excluded. Finally, individuals with a known history of hypersensitivity to Holixer™ or any of its components were excluded.

Participant and Public Involvement

The participants and the public were not involved in developing the research question, study design, recruitment, reporting, or dissemination plans. Eligible individuals were included; however, participants did not receive personal notifications of the study findings. However, they could request and access the results. The outcomes will also be shared through scientific publications, based on the collected data.

2.5. Intervention

The intervention Holixer™ (containing *Ocimum tenuiflorum* extract) was supplied by Natural Remedies (Bangalore, India). Holixer™ is a patent-pending (202241023540) hydroalcoholic extract derived from the leaves' rich aerial parts of *Ocimum tenuiflorum*. It is manufactured in a good manufacturing practice (GMP)-approved facility and phytochemically standardized to $\geq 5\%w/w$ (by High-Performance Liquid Chromatography) of the *Ocimum* Bioactive Complex. It complies with the United States Pharmacopeia (USP) requirements for microbial, heavy metal, residual pesticide, aflatoxin, and residual solvent limits for dietary supplements [7].

The Holixer™ and placebo capsules were identical in appearance and matched for color, shape, size, smell, and taste. The intervention contained 250 mg *Ocimum tenuiflorum* extract per capsule. Placebo capsules contained microcrystalline cellulose. Both the intervention and placebo were administered at a GMP-certified facility. All participants were instructed to take 2 capsules once daily for 8 weeks.

Capsule adherence was evaluated by asking participants to bring the whole bottle while coming on the visit and asked whether the IP was taken daily in the prescribed way, lost some of the capsules/tablets, or left in a box. Any inconsistencies in the number of capsules or tablets returned were explained. The number of capsules/tablets returned by the participants was recorded and used to calculate compliance percentage.

2.6. Outcome measures

2.6.1. Primary outcome measure

The primary efficacy endpoint was CAP rate change (Total CAP rate decrease, CAP A1 rate increase, and CAP A2+A3 rate) between the two groups.

A cyclic alternating pattern (CAP) cycle is defined as the combination of a Phase A period followed by a subsequent Phase B period. CAP rate was calculated as the ratio (expressed as a percentage) of total CAP time to total non-rapid eye movement (NREM) sleep time. Phase A periods were further subdivided into three subtypes. Subtype A1 consists of synchronized events, such as sleep spindles, K-complexes, and delta bursts, which exert minimal influence on autonomic and somatomotor activity. Subtype A2 represents a mixed pattern of synchronized and desynchronized EEG events, with an intermediate impact on autonomic and somatomotor functions. Subtype A3 is predominantly characterized by desynchronized EEG events, including microarousals, and is associated with substantial autonomic and somatomotor activations.

The CAP rates, phases of CAP, and other structural indices were assessed using Nocturnal Polysomnography (NPSG). NPSG recordings were obtained from bedtime to awakening in the morning and from continuous audiovisual IR monitoring of the subjects. Sleep stages were manually scored in 30sec epochs according to the standardized criteria outlined by the American Academy of Sleep Medicine (AASM)- Manual for the Scoring of Sleep and Associated events [11]. Electrode placement for EEG was performed according to the International 10–20 System. The ALICE 6® computerized system, Phillips Respironics Inc. Murrayville, USA, was used for the NPSG.

2.6.2. Secondary outcome measures

The secondary objective of this study was to compare the effects of Holixer™ and placebo on changes from baseline to eight weeks in key sleep architecture indices, including sleep efficiency, wakefulness after sleep onset (WASO), rapid eye movement (REM) sleep latency, and REM sleep duration. In addition, changes in psychological and sleep quality

outcomes, as measured by validated questionnaires, such as the Perceived Stress Scale (PSS), Insomnia Severity Index (ISI), Pittsburgh Sleep Quality Index (PSQI), and Restorative Sleep Questionnaire (RSQ W), were used to assess stress and sleep quality. Additional objectives included the assessment of adverse events throughout the study period and evaluation of participants' compliance with the assigned study treatments.

- **Sleep changes as measured by a Actiwatch**

Actigraphy is a non-invasive method for measuring the sleep-wake cycle in humans. Actigraphy readings for seven days were used to evaluate average sleep onset latency, wakefulness after sleep onset, and sleep efficiency in participants' own home settings for a period of seven days before and after the intervention.

- **Insomnia Severity Index (ISI)**

The ISI is a Likert-based paper pencil validated questionnaire used to screen adults for insomnia. It has five items. The total score is a sum of individual item scores. These scores are then categorized ranging from clinically insignificant or severe insomnia [12] [13].

- **Pittsburgh Sleep Index Questionnaire (PSQI)**

PSQI is 19-self reported questionnaire that is subcategorized into subjective sleep quality and quantity, daytime functioning, and sleeping medication. A score greater than five indicated disturbed sleep [14].

- **Perceived stress scale**

The PSS is a widely used and validated self-report questionnaire that measures the degree to which situations in one's life have been evaluated as stressful over the last month. The PSS scores ranged from 0 to 40, with higher scores indicating higher perceived stress. An

online version of the PSS was completed at baseline and weeks 4 and 8. A score greater than 14 indicates moderate-to-severe stress [15] [16].

- **Restorative sleep questionnaire, weekly version**

The RSQ-W is a validated scale for measuring refreshing quality of sleep [17]. It has nine items, with answers ranging from 1 to 5. Some items were reverse scored. The total score is the average score based on all nine items and rescaled to a 0-100 scale, using the following transformation:

$$\text{RSQ-W Total Score} = (\text{RSQ-W average score across completed items} - 1) \times 25.$$

- **Adverse events**

Tolerability of capsule intake was assessed every week via a telephonic question inquiring about the adverse effects believed to be associated with capsule intake. Participants were also asked to contact the researchers if they experienced any adverse effects.

2.7. Sample size calculation

The primary objective of this study was to estimate the change in the CAP rate at 8 weeks from baseline between the HolixerTM and placebo groups. On the basis of a single outcome variable, an a priori power analysis was performed to estimate the required sample size. In a previous trial on paradoxical insomnia, the SD from previous studies for the CAP rate difference was ~6.8.

With a power of 90% and a type-one error rate (alpha) of 5%, the number of participants needed per group to find a treatment effect was estimated to be 30. After allowing for an approximate dropout rate of 15 %, 35 participants were recruited per group.

2.8. Statistical analysis

All statistical analyses were performed using the SPSS version 26.0 (IBM Corp., Armonk, NY, USA). All analyses were performed on both intent-to-treat (ITT) and per-protocol (PP) datasets. Both intent-to-treat (ITT) and per-protocol (PP) analyses were also performed. As there were no dropouts and all randomized participants adhered to the protocol, the ITT and PP populations were identical. Continuous variables are summarized as mean $\pm$ (SD). Baseline demographic data and scores were compared between the two groups of HolixerTM and Placebo. The normality of the data distribution was assessed using the Shapiro–Wilk test. Paired t-tests were used for within-group comparisons. Between-group differences were analyzed using independent sample t-tests. Pearson’s chi-squared test was used to compare categorical data. All tests were two-tailed, and statistical significance was set at $p < 0.05$. Changes in the scores from baseline were calculated and expressed as the mean and percentage change from baseline.

3. RESULTS

3.1. Study population

Demographic information and baseline polysomnography and actigraphy information

In total, 328 volunteers were screened for eligibility. Of these, 197 did not meet the inclusion criteria and 61 declined to participate. Ultimately, 70 participants were enrolled and randomized, with 35 assigned to the HolixerTM group and 35 to the placebo group. All 70 participants completed the study [Fig. 2]. The baseline demographic characteristics were comparable between the groups, and no statistically significant differences were observed in the baseline scores between the HolixerTM and placebo groups [Table 1].

3.2. Outcome measures

3.2.1. Primary outcome measures: Polysomnography microstructure variables

After eight weeks of intervention, the mean total CAP rate remained unchanged in both groups. However, a significant increase of 95% in the CAP A1 rate was observed in the HolixerTM group ($p = 0.03$), whereas the placebo group showed a non-significant decrease of 28.57% ($p = 0.29$). A group comparison revealed a statistically significant increase in CAP A1 rates at week 8 in the HolixerTM group compared with the placebo ($p = 0.007$). In contrast, the mean changes in CAP A2 + A3 rates remained similar post-intervention, and no significant between-group differences were observed [Table 2].

3.2.2. Secondary outcome measures

a. Polysomnography variables

As shown in Table 2, no statistically significant between group differences were observed in total sleep time, stage 3 sleep, REM duration, or REM latency ($p > 0.05$). Although macrostructural parameters such as total sleep time, stage 3 sleep, REM duration, and REM latency did not differ significantly between the groups, the marked increase in CAP A1 rates in the HolixerTM group highlights meaningful improvements in the sleep microarchitecture.

b. Actigraphy variables

- Sleep onset latency

As shown in Table 2, a non-significant decrease in sleep onset latency was observed in the HolixerTM group compared to the baseline ($p = 0.24$). In contrast, the placebo group demonstrated a significant increase in sleep onset latency ($p = 0.005$). A group

comparison at week 8 revealed a statistically significant difference, favoring the Holixer™ group ($p = 0.02$).

- Sleep efficiency

As shown in Table 2, sleep efficiency increased significantly by 5.7% from baseline to week 8 in the Holixer™ group ($p = 0.03$). In the placebo group, a non-significant increase of 0.23% was observed over the same period ($p = 0.89$). A group comparison of the mean scores at week 8 showed a trend toward significance ($p = 0.07$).

- Wakefulness After Sleep Onset

As shown in Table 2, wakefulness after sleep onset decreased by 20% in the Holixer™ group, showing a trend toward significance ($p = 0.07$). In contrast, the placebo group exhibited an insignificant reduction of 6.21% ($p = 0.38$). A group difference of the mean wakefulness time at week 8 also indicated a trend toward statistical significance ($p = 0.08$).

c. Perceived Stress Scale

As shown in Table 3, the Holixer™ group showed a statistically significant reduction in Perceived Stress Scale (PSS) scores of 25.64% at week 4 ($p = 0.0001$) and 21.59% at week 8 ($p = 0.0004$) compared with baseline. The placebo group also demonstrated significant decreases of 13.81% at week 4 ($p = 0.003$) and 16.08% at week 8 ($p = 0.003$). However, the group differences in the PSS scores were not statistically significant ($p = 0.5$).

d. Insomnia Severity Index (ISI)

As shown in Table 3, the Holixer™ group exhibited statistically significant reductions in Insomnia Severity Index (ISI) scores of 47.22% at week 4 ($p = 0.009$) and 50.46% at week 8 ($p = 0.005$) compared with baseline. The placebo group showed a non-significant decrease of 33.67% at week 4 ($p = 0.09$) and a significant decrease of 32.33% at week 8 ($p = 0.04$). The group differences in mean ISI scores reached statistical significance from week 4 ($p = 0.002$) and remained significant at week 8 ($p = 0.005$).

e. Pittsburgh Sleep Quality Index (PSQI)

As shown in Table 3, the Holixer™ group showed statistically significant reductions in Pittsburgh Sleep Quality Index (PSQI) scores of 27.72% at week 4 ($p = 0.001$) and 33.70% at week 8 ($p = 0.007$), compared with baseline. In contrast, the placebo group did not exhibit statistically significant reductions in PSQI scores at either time point. However, the between-group differences in PSQI scores were not statistically significant.

f. Restorative sleep questionnaire, weekly version

As demonstrated in Table 3, there were no statistically significant between-group differences in RSQ-W scores.

3.2.3. Intake of supplements

Capsule bottles with the remaining capsules were returned in the Week 8 assessment. The compliance percentages were calculated based on the remaining capsules. Based on these details, 94.18% and 95% of patients were in the placebo and Holixer™ groups, respectively.

3.2.4. Adverse effects

No major treatment-related adverse events were reported in either the placebo or the Holixer™.

4. DISCUSSION

This 8-week randomized double-blind placebo-controlled study demonstrated that supplementation with *Ocimum tenuiflorum* extract (Holixer™) was associated with significant improvements in sleep microarchitecture and quality in stressed individuals. Polysomnographic analysis revealed a statistically significant increase in cyclic alternating pattern (CAP) A1 rates in the Holixer™ group compared with the placebo group, corresponding to an approximate 95% increase from baseline, whereas the placebo group exhibited a 28% decrease over the same period. One of the most noteworthy findings was the robust increase in cyclic alternating pattern (CAP) A1 rates following Holixer™ intake. CAP A1 activity is considered to be a hallmark of stable non-REM sleep and reduced vulnerability to arousal.

Significant improvements in the subjective sleep quality were observed. The Insomnia Severity Index (ISI) scores decreased significantly in the Holixer™ group relative to the placebo group, reflecting a 50% reduction in overall distress due to sleep problems compared with a 32% reduction in the placebo group. Similarly, the Pittsburgh Sleep Quality Index (PSQI) demonstrated a significant decrease following Holixer™ intake, whereas the reduction in the placebo group did not reach statistical significance.

Objective sleep parameters assessed using actigraphy (Actiwatch) further corroborated these findings. Holixer™ intake was associated with a significant reduction in sleep onset latency compared to placebo. In addition, actigraphic data indicated a 5.7% increase in sleep efficiency and 20% improvement in wakefulness after sleep onset (WASO).

Analysis of sleep efficiency and WASO revealed a trend toward significance between the groups, suggesting potential benefits that warrant further investigation in larger cohorts.

Collectively, these findings highlight the efficacy of Holixer™ in enhancing both the subjective and objective dimensions of sleep quality, with a particular improvement in CAP A1 dynamics—a marker of sleep stability and quality.

Sleep has traditionally been divided into two broad states: rapid eye movement (REM) and non-REM (NREM). The American Academy of Sleep Medicine (AASM) scoring manual is further classified into three distinct stages [18]. Given that individuals with mild to moderate stress are unlikely to exhibit substantial alterations in macrostructural polysomnographic parameters, the present study specifically focused on evaluating the sleep microarchitecture. Our findings confirmed this hypothesis, as no significant differences were observed in the distribution of the NREM and REM sleep stages between the groups. Notably, this aligns with prior evidence in clinical populations. For example, a study in patients with Parkinson's disease reported an absence of macrostructural changes in sleep, suggesting that even in neurological conditions with known sleep involvement, macrostructural parameters may remain unaffected [19].

The authors hypothesized that moderate to high perceived stress is associated with poor sleep quality, potentially mediated by disturbances in the sleep microarchitecture. The cyclic alternating pattern (CAP) is a well-recognized biomarker of sleep microarchitecture and is composed of alternating Phase A and Phase B activities [19]. CAP occurs across all stages of NREM sleep and is closely associated with sleep stability and depth [20]. Importantly, CAP contributes to arousal regulation in both physiological and pathological conditions.

In the present study, no significant differences in overall CAP rates were observed between the groups. Several factors may have accounted for this finding. A primary consideration is that the stressed population under investigation demonstrated relatively similar baseline CAP rates before intervention. Although elevated CAP activity is typically anticipated in individuals under heightened stress, this increase was not evident in this cohort. Consequently, the absence of baseline elevation may explain the lack of significant changes in the total CAP rates.

It is important to examine CAP cycles at a finer level of resolution. CAP consists of Phase A divided into three subtypes: A1, A2, and A3 [21]. CAP A1 is associated with deeper and more efficient slow-wave sleep (SWS), supporting processes, such as phase–amplitude coupling, synaptic plasticity, and the brain’s efforts to maintain stable sleep. Functionally, CAP A1 is linked to the establishment and maintenance of consolidated sleep, and to the reduction of arousal and fragmentation. CAP A2 represents a transitional stage between A1 and A3, whereas CAP A3 is frequently associated with increased sleep fragmentation and arousals [22].

A substantial body of evidence indicates that decreases in CAP A1, accompanied by increases in CAP A2 and A3, correlate with sleep instability, arousal, and reduced restorative sleep properties. Furthermore, diminished CAP A1 rates have been associated with both sleep and nonsleep cognitive disorders [23]. In the present study, we observed a 95% statistically significant improvement in CAP A1 rates in the Holixer™ group compared with the placebo group. These findings suggest that Holixer™ supplementation enhances sleep quality and stability by reducing sleep fragmentation and arousal. To our knowledge, no published study to date has investigated the effects of herbal interventions

on the microarchitecture of sleep across CAP subtypes A1, A2, and A3, making these results novel and clinically relevant.

This is the first study to report the benefits of Holixer™ on CAP A1 rates. Notably, situations in which the overall CAP rates remain unchanged, while CAP subtypes show meaningful alterations, have been described previously. For example, Simor et al. (2013) reported that individuals with nightmares exhibited unaltered total CAP rates but demonstrated a decrease in CAP A1 and increase in CAP A3, underscoring that subtype-specific CAP dynamics may be more critical than total CAP in interpreting sleep stability and fragmentation [23].

In addition to polysomnography, the present study incorporated actigraphy (Actiwatch) to assess objective sleep outcomes including sleep onset latency, sleep efficiency, and wakefulness after sleep onset (WASO). Actigraphy is considered a valid and reliable research tool for objective assessment of sleep quality and is strongly correlated with polysomnographic findings [24]. In the Holixer™ group, sleep onset latency was significantly reduced compared with placebo, with participants falling asleep approximately seven minutes faster. Furthermore, sleep efficiency increased by 5.7%, corroborating earlier findings from a study using a Fitbit device that reported a 3.8% increase in sleep efficiency following Holixer™ intake [7]. Together, these findings from two independent modalities support the robustness of Holixer's effects on sleep efficiency.

Finally, WASO significantly decreased in the Holixer™ group, with a trend toward significance in between-group comparisons. This pattern complements CAP findings, suggesting reduced nocturnal arousal, improved sleep stability, and decreased

fragmentation, as evidenced by the substantial increase in CAP A1 rates. Collectively, these results indicate that HolixerTM supplementation not only enhances sleep microarchitecture but also translates into measurable improvements in objective sleep quality.

Sleep quality is generally characterized by four key attributes: sleep efficiency, sleep latency, wakefulness after sleep onset (WASO), and total sleep duration [25]. Although the total sleep time did not differ significantly between the groups, HolixerTM supplementation was associated with improvements in three major pillars of sleep quality, sleep efficiency, sleep onset latency, and WASO. This may translate into the functioning of the next day.

Importantly, subjective perceptions of sleep quality were also evaluated using the Insomnia Severity Index (ISI) and the Pittsburgh Sleep Quality Index (PSQI). Participants in the HolixerTM group reported substantial improvements in perceived sleep quality, consistent with objective outcomes measured using both polysomnography and actigraphy. This convergence of subjective and objective evidence strengthens the conclusion that HolixerTM supplementation provides meaningful improvements in sleep quality in individuals experiencing stress.

Limitations & Strengths

The major limitation of this study was the inclusion of moderately stressed individuals with CAP rates comparable to those observed in the general population. Although elevated CAP rates are typically anticipated under higher stress conditions, such increases

were not evident in this cohort. The inclusion of individuals with higher perceived stress levels may have resulted in greater CAP activity and more pronounced sleep instability, thereby providing clearer group differences. Although the sample size for objective assessments was adequate, subjective measures such as the Perceived Stress Scale (PSS) and Restorative Sleep Questionnaire (RSQ) may require larger cohorts to reliably demonstrate between-group differences. In addition, the study did not evaluate next-day cognitive performance, which could plausibly result from the enhanced memory consolidation associated with increased CAP A1 rates.

This study had several strengths. This is the first randomized, double-blind, placebo-controlled clinical trial to investigate the effects of a standardized *Ocimum tenuiflorum* extract (HolixerTM) on the microarchitecture of sleep, specifically the cyclic alternating pattern (CAP) subtypes. Second, the use of gold-standard polysomnography (PSG) along with validated actigraphy (Actiwatch) provided a comprehensive assessment of sleep microstructure, enhancing the robustness of the findings. Finally, the novelty of assessing CAP subtypes in a stressed population provides valuable insights into the mechanisms underlying sleep restoration and sets a precedent for future research on herbal interventions in sleep medicine.

5. CONCLUSION

In conclusion, this is the first study to evaluate the effects of an herbal intervention, HolixerTM (*Ocimum tenuiflorum* extract), on sleep microarchitecture. The findings demonstrated that eight weeks of HolixerTM supplementation at a daily dose of 250 mg

was associated with improvements in sleep quality, stability, and consolidation, as reflected by increased CAP A1 rates, reduced fragmentation, and fewer arousals. Holixer™ supplementation was well tolerated with no major treatment-related adverse events. Importantly, improvements in objective sleep parameters paralleled enhanced subjective perceptions of sleep quality, supporting Holixer™ as a promising, safe, and effective botanical intervention for stress-related sleep disturbances.

Data availability statement

Data supporting the conclusions of this article will be provided by the authors without undue reservation upon request.

Institutional Ethics Committee Statement

The studies involving human participants were reviewed and approved by the International Institute of Sleep Sciences. All participants provided written informed consent to participate in this study.

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Author Contributions

All three authors, Dr. Abhijit Deshpande, Dr. Nushafreen Irani, and Dr. Pearl Irani, were involved in the conceptualization, methodology, design, conduct, reporting, investigation,

writing, review, and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

This study was funded by Natural Remedies Pvt. Ltd. The funder was not involved in the design of the research study, data analysis, or the writing of the report. Dr. Abhijit Deshpande, Dr. Nushafreen Irani, and Dr. Pearl Irani are employees of the Ethicare Clinical Trial Services (OPC) Pvt. Ltd., a clinical research organization.

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Declaration

The Generative AI tool (ChatGPT – GPT-4) was used for grammar refinement.

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Table.1 Baseline demographic and clinical characteristics

		Placebo	Holixer TM	p value
Age (years)	Mean	35.77	34.54	0.44
	SD	8.54	10.31	
Sex	Female	14	18	0.34
	Male	21	17	
Height (cm)	Mean	161.23	162.2	0.57
	SD	9.12	11.28	
Weight (kg)	Mean	69.2	67.49	0.9
	SD	12.17	14.11	
BMI (kg/m ²)	Mean	26.57	25.74	0.3
	SD	4.25	5.18	
CAP rate (%)	Mean	30.72	32.2	0.63
	SD	12.5	11.16	
Cap A1 rate (%)	Mean	1.89	1.67	0.7
	SD	2.79	2.05	
A2+ A3 rate (%)	Mean	28.83	30.52	0.57
	SD	13.35	11.52	
SOL (mins)	Mean	10.42	13.33	0.14
	SD	5.72	10.24	
SE (%)	Mean	80.25	78.41	0.39
	SD	6.42	11.13	
WASO (mins)	Mean	81.29	83.48	0.83
	SD	34.33	50.43	

CAP Rate (%) = (Total duration of CAP phases in NREM sleep / Total duration of NREM sleep) x 100

CAP A1 Rate (%) = (Total duration of CAP A1 phases in NREM sleep / Total duration of NREM sleep) x 100

CAP A2 + A3 Rate (%) = ((Total duration of CAP A2 phases + Total duration of CAP A3 phases) in NREM sleep / Total duration of NREM sleep) x 100

BMI=weight(kg)/[height(m)]²

Sleep Efficiency = (Total Sleep Time / Total Time in Bed) x 100.

Table.2 Changes in polysomnography and actigraphy variables (microarchitecture of sleep)

		Placebo		Holixer TM						p value Placebo vs Holixer TM
Polysomnography		Baseline	Week 8	p value	% change	Baseline	Week 8	p value	% change	
CAP rate (%)	Mean	30.72	30.18	0.84	1.76	32.2	33.43	0.63	3.82	0.18
	SD	12.5	9.97			11.16	10.11			
Cap A1 rate (%)	Mean	1.89	1.35	0.29	28.57	1.67	3.26	0.03	95.21	0.007
	SD	2.79	1.22			2.05	3.88			
A2+ A3 rate (%)	Mean	28.83	28.84	0.99	0.03	30.52	30.17	0.89	1.15	0.59
	SD	13.35	10.39			11.52	10.27			
REM Latency										
(mins)	Mean	81.76	86.23	0.66	-5.47	93.76	92.21	0.87	1.65	0.57
	SD	36.86	48.7			39.2	40.12			
REM Duration										
(mins)	Mean	103.1	100.4	0.75	2.62	96.79	95.99	0.94	0.83	0.61
	SD	31	41.3			31.64	30.31			
Stage 3 NREM										
(mins)	Mean	100.94	95.19	0.51	5.7	105.5	100.63	0.39	4.62	0.52
	SD	38.25	34.9			41.08	32.36			
TST (mins)	Mean	395.53	381.61	0.32	3.52	388.79	378.71	0.4	2.59	0.84
	SD	46.12	68.24			53.15	52.51			
Actigraphy										
SOL (mins)	Mean	10.42	14.69	0.005	40.97	13.33	10.82	0.24	18.83	0.02
	SD	5.72	6.74			10.24	7.28			
SE (%)	Mean	80.25	80.44	0.89	0.24	78.41	82.88	0.03	5.7	0.07
	SD	6.42	5.9			11.13	5.33			
WASO (mins)	Mean	81.29	76.24	0.38	6.21	83.48	66.78	0.07	20.00	0.08

SD	34.33	2.99	50.43	20.72
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CAP Rate (%) = (Total duration of CAP phases in NREM sleep / Total duration of NREM sleep) x100

CAP A1 Rate (%) = (Total duration of CAP A1 phases in NREM sleep / Total duration of NREM sleep) x 100

CAP A2 + A3 Rate (%) = ((Total duration of CAP A2 phases + Total duration of CAP A3 phases) in NREM sleep / Total duration of NREM sleep) x 100

Table.3 Changes in self-reported questionnaires

												p value	
												Placebo vs	
Placebo						Holixer™						Holixer™	
					Week	p			Week	Week		Week	Week
		Baseline	Week 4	p value	8	value	Baseline	4	p value	8	p value	4	8
PSS	Mean	18.83	16.23	0.003	15.8	0.003	19.06	14.17	0.0001	14.94	0.0004	0.07	0.5
	SD	3.36	3.73		4.76		2.83	5.61		5.92			
ISI	Mean	8.57	5.69	0.09	5.80	0.04	6.17	3.26	0.009	3.06	0.005	0.002	0.005
	SD	6.26	3.68		4.92		5.79	2.85		2.85			
PSQI	Mean	5.91	4.09	0.09	4.37	0.2	5.26	3.8	0.001	3.49	0.007	0.6	0.12
	SD	3.33	2.54		2.61		3.18	2.19		2.08			
RSQ-W	Mean	63.55	77.55	0.006	77.3	0.005	74.88	79.6	0.15	79.81	0.16	0.41	0.46
	SD	17.79	14.81		13.53		14.78	12.34		14.37			

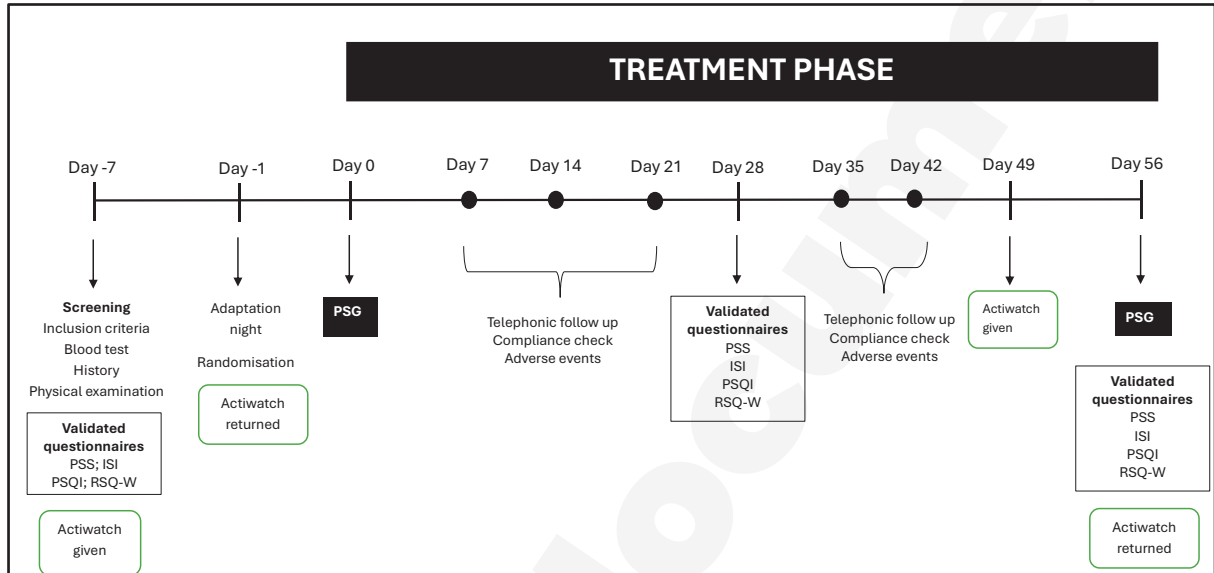


Figure1. Participant visits and assessments

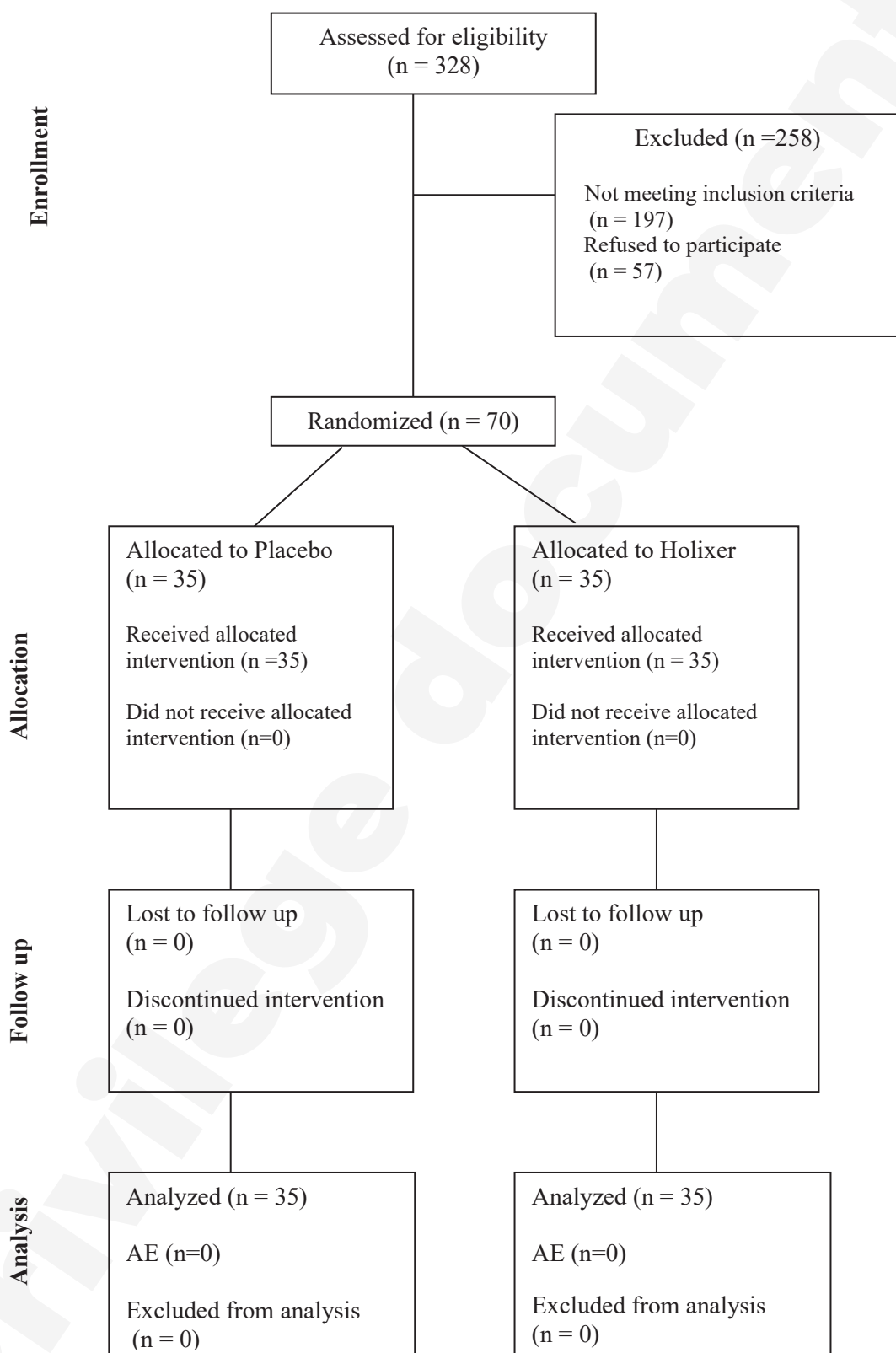


Figure.2. Participants CONSORT flow diagram.

LIST OF ABBREVIATIONS

AHI	Apnea/ Hypopnea Index
AASM	American Academy of Sleep Medicine
AE	Adverse Event
AHI	Apnea Hypopnea Index
CAP	Cyclic Alternating Pattern
CBC	Complete Blood Count
CMPs	Complete Metabolic Panel
CRA	Clinical Research Associate
CSA	Case Record form
CAD	Coronary Artery Disease
CAP	Cyclic Alternative Pattern
CHD	Coronary Heart Disease
CNS	Central Nervous System
ECG	Electrocardiogram
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electrooculogram

H&P	History and Physical
HADS	Hospital Anxiety Depression Scale
ICF	Inform Consent form
ICH	International Conference on Harmonization
ICSD	International Classification of Sleep Disorders
IEC	Independent Ethics Committee
ISI	Insomnia Severity Index Scale
IP	Investigational Product
NPSG	Nocturnal Polysomnogram
NREM	Non-Rapid Eye Movement
PSQI	Pittsburgh Sleep Quality Index
PSS	Perceived Stress Scale
PLMD	Periodic Limb Movement Disorder
REM	Rapid Eye Movement
RLS	Restless Leg Syndrome
RSQ-W	Restorative Sleep Questionnaire weekly
SAE	Serious Adverse Event

SE	Sleep Efficiency
SOL	Sleep Onset latency
TBT	Total Bedtime
TST	Total Sleep Time
WASO	Wake After Sleep Onset