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**Original Research Article**

# A randomized, double-blind, parallel and placebo-controlled clinical study to evaluate the efficacy and safety of KaraCalm™: a dietary supplement to support sleep and manage stress

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## ABSTRACT

**Background:** KaraCalm™ is a novel polyherbal formulation obtained from the combination of *Valeriana officinalis* extract, *Passiflora incarnata* extract, *Ocimum sanctum* extract, *Ziziphus jujuba* extract, *Rosmarinus officinalis* extract, and *Nigella sativa* extract. The objective of the present investigation was to assess the efficacy and safety of KaraCalm™ to manage stress and improve sleep in healthy subjects in a randomized, double-blind, placebo-controlled clinical study.
**Methods:** A total of 60 healthy volunteers were randomized into two groups, with 30 subjects in the KaraCalm™ group and 30 in the placebo group. Participants were asked to take KaraCalm™ 500 mg or placebo once daily for 56 days. As primary outcomes, sleep analysis was performed by using Actiwatch, while stress level was evaluated with the Perceived Stress Scale (PSS) scores from baseline to the end of the study period. Serum cortisol, and hs-CRP from baseline to the end of the study period were assessed as secondary endpoints.
**Results:** An increase in overall sleep quality was observed in the KaraCalm™ group compared to the placebo as measured by total sleep time, onset latency, wake after sleep onset minutes, and number of awakenings. There was also a reduction in PSS scores in the KaraCalm™ group from baseline to the end of the study, indicating reduced stress levels. A significant reduction in Serum cortisol and hs-C-reactive protein (CRP) levels in the KaraCalm™ group from baseline to the end of the study further supported the effectiveness of KaraCalm™ in reducing stress. There was no significant change in the safety analyses of the patients in the intervention group when assessed from the start of the study to the end.
**Conclusions:** KaraCalm™ can be considered a safe and effective dietary herbal Supplement to reduce stress and improve sleep quality.

**Keywords:** KaraCalm™, Polyherbal formulation, Sleep quality, Stress reduction, Clinical trials

## INTRODUCTION

Stress responses are an outgrowth of the primitive "fight or flight" instinct, initially designed to defend against imminent physical danger. Chronic exposure to stress prolongs the fight-or-flight mechanism, leading to physiological changes related to increases in heart rate, blood pressure, and blood sugar levels, as well as reduced

blood flow to the digestive system. These changes are symptomatic of an overactive sympathetic nervous system, which increases the production of stress hormones such as cortisol and epinephrine.[1] Excessive secretion of these hormones, particularly cortisol, affects neural structures such as the hippocampus negatively, causing memory deficits and, negatively affecting sleep through the sympatho-adreno-medullary (SAM) and hypothalamic-pituitary-adrenal (HPA) axis.[2]

As per the Diagnostic and Statistical Manual of Mental Disorders (DSM), fourth edition, sleep disorders occur primarily in the presence of acute psychological, social, and medical stress. This was demonstrated by a study on 345 patients evaluated for sleep disorders.[3] Therefore, there is a strong correlation between stress and sleep. Stress triggers psychophysiological responses by the activation of the HPA system, which is not compatible with regular sleep resulting in the outbreak of sleep disorders. The latter represent a broad spectrum of categorical disorders, including sleep-related breathing disorders, insomnia, central hypersomnolence disorders, parasomnias, circadian sleep-wake rhythm disorders, and sleep-related movement disorders.[4] Sleep disturbances result in irritability, low energy and motivation, daytime sleepiness, impaired cognitive function, and physical discomfort.[4] In fact, sleep is crucial for maintaining brain homeostasis, promoting brain plasticity, and sustaining mental and physical well-being. Therefore, sleep disorders can lead to allostatic overload, disrupting neural plasticity, immune function, and endocrine pathways and potentially exacerbating mental health and physical disorders.[5] For these reasons, several efforts have been made in recent years to develop sleep disorders medicine leading to the impressive growth of sleep clinics with qualified sleep specialists offering professional care to those experiencing various sleep disorders.[6]

Confirming the steady increase in sleep quality-related disorders is the constant increase in sleep medication whose prescription has seen a 293% increase in the number of outpatient visits in the United States.[4] This results in an exceedingly high intake of benzodiazepine receptor agonists, second-generation benzodiazepines or Z-drugs (zolpidem, zopiclone, or zaleplon), and many other sedative medications.[4] Concerning sleep quality, either benzodiazepines or Z-drugs shorten sleep onset latency; however, they are less effective in improving sleep efficiency or total sleep time. Moreover, a large number of studies have shown serious adverse effects related to these drugs, including next-day hangovers, cognitive or memory impairment, excessive daytime sleepiness, traffic accidents, abuse, addiction, and other abnormal sleep-related patterns, such as compulsive nighttime eating.[7] Considering these issues, safer options such as the use of botanicals that act as adaptogens and/or sedatives and calmatives are needed.

The term "adaptogen" refers to botanical substances that enhance the body's nonspecific response to stress and facilitate recovery from it. Brekhman coined the term adaptogen; for a botanical to be an adaptogen it must possess four general properties: it must be harmless to the host; it must have a general, rather nonspecific effect; it must increase the recipient's resistance to a variety of physical, chemical, or biological stresses; and it must act as a general stabilizer or normalizer.[8] Several vegetal species have been historically employed to reduce stress and ameliorate sleep quality, among these: *Valeriana officinalis L., Humulus lupulus L., Passiflora incarnata L., Matricaria chamomilla L., Ocimum sanctum L., Ziziphus jujuba Mill., Piper methysticum G.Forst., Rosmarinus officinalis L., and Nigella sativa* L.[1,9] A recent meta-analysis showed that *Valeriana officinalis* could be an effective and safe herbal product to induce sleep and prevent related disorders.[10] Similarly, *Ocimum tenuiflorum L.* extract (also known as *Ocimum sanctum*) supplementation in adults reduces stress symptoms and improves sleep quality, while a randomized study on 68 university students highlights the ability of *Rosmarinus officinalis* to improve sleep quality and reduce anxiety and depression.[11-13] Based on this background a novel polyherbal supplement was formulated, KaraCalm™. This product is a combination of *Valeriana officinalis* extract, *Passiflora incarnata* extract, *Ocimum sanctum* extract, *Ziziphus jujuba* extract, *Rosmarinus officinalis* extract, and *Nigella sativa* extract. This is a randomized, double-blind, placebo-controlled clinical study to assess the efficacy and safety of KaraCalm™ to manage stress and improve sleep in healthy subjects.

## METHODS

### *Study design*

This is a randomized, double-blind, parallel, placebo-control study to assess the efficacy and safety of KaraCalm™ to manage stress and improve sleep in adults conducted at the Department of Psychiatry of Vatsalya Hospital between 18 July 2022 and 28 February 2023. The primary endpoint of this study was to assess the change in Sleep analysis by using ActiWatch and perceived stress scale (PSS) scores from baseline to the end of the study period. The secondary endpoints were the evaluation of serum cortisol and hs-CRP from baseline to the end of the study period. The study was performed on healthy, disease-free adults.

### *Inclusion criteria*

The inclusion criteria for the study are: male and female healthy adult subjects ranging in age from 18 to 54 years; adult subjects willing to provide written informed consent; free of psychiatric conditions other than mild stress; and could read and write English.

### *Exclusion criteria*

The exclusion criteria for the study are: subjects with insomnia, sleep disorders, or chronic stress; excessive

alcohol consumption (more than 2 standard pegs/day); subjects with diagnosed hypertension and other diseases of the cardiovascular system; subjects with diagnosed liver diseases, kidney diseases, psychiatric diseases, epilepsy and/or any other relevant diseases; subjects unwilling or unable to adhere to the study protocol; subject participating in another clinical trial or received any IP within 90 days before visit 1 (screening); retraction of the written informed consent; subjects currently taking medications other than oral contraceptive pills; participants on hepatotoxic medications including antitubercular medication, antiviral medication, paracetamol; pregnant, attempting to conceive, or lactating women; individuals with acute narrow-angle glaucoma, prostate hypertrophy, cardiovascular, endocrine or renal disease, or another chronic disease that could affect stress/anxiety or restrict normal, daily function were also ineligible to participate in the study; and individuals who currently, or in the past 6 months, suffered from any diagnosed mental-health disorders (as assessed by the mini international neuropsychiatric interview 6.0) or were taking a psychotropic medication or other herbal preparation were also excluded from participating in the study.

### *Ethical consideration*

The study was performed following the current version of the Declaration of Helsinki. The trial agreed with the international conference on harmonisation (ICH) guidelines on good clinical practice (GCP) and India's rules and regulations. The study was performed under strict compliance with the requirements of the Indian regulations for carrying out herbal and Ayurvedic clinical trials and ASU-GCP. Also, ICH guidelines for good clinical practice (ICH-GCP) issued by the U.S. Department of Health and Human Services were followed wherever applicable. The trial was registered with the clinical trials registry (CTRI) and hosted at the ICMR's National Institute of Medical Statistics as per the Drugs Controller General of India (DCGI) mandate. Each subject was provided with a subject information sheet (SIS) with detailed procedures involved in the study (aims, methodology, potential risks, and anticipated benefits). The investigator explained these to each subject, who was given ample time to consider the information presented. The study protocol and its amendments and the patient information sheet(s) were reviewed and approved by the Vatsalya Ethics Committee on 15 May 2022. The trial was registered on 01 June 2022, and the study was issued with the following registration number: CTRI/2022/07/043761.

### *Participants and randomization*

Seventy-two subjects were assessed for eligibility. As the tested medications comprised ingredients known for human consumption, specific attention was given to subjects who might not be aware of their specific allergy to these constituents or herbs in general and might develop an allergy during the trial. Those with known allergies to KaraCalm™ products were excluded from the trial. At the end of the screening, 60 patients were selected for the study and randomized after the investigators had obtained written consent. The randomization codes were generated by computer using permuted block design, and the block size selected was known only to the statistician until the statistical analysis was completed. The sponsor's designee maintained the randomization code list, which was provided to the CRO and study sites in a concealed envelope. The site coordinator informed the sponsor's manager after assigning the randomization code to eligible subjects. At no time during the study was the code violated, or was a subject given a non-blinded study product. All study subjects, investigators, and sponsor personnel remained blinded to the study medication assignment, but restricted access to codes in an emergency is available through the sponsor's designated person for randomization/blocking. Following the randomization, the 60 selected subjects were distributed equally into two study arms of KaraCalm™ (group A=30 subjects) or placebo (group B=30 subjects). The total duration of the study was 56 days. Each subject needed to report nine times at the clinical center during visits 1 to 9, including on screening and randomization days. Six subjects from group A and six from group B withdrew due to a loss of follow-up. The final statistical analysis and results were depicted for 48 participants at the end of study (Figure 1).

### *Study intervention*

KaraCalm™ 500 mg capsule administered orally once daily. KaraCalm™ is a proprietary product containing the combination of well-known natural ingredients *Valeriana officinalis* extract, *Passiflora incarnata* extract, *Ocimum sanctum* extract, *Ziziphus jujuba* extract, *Rosmarinus officinalis* extract, and *Nigella sativa* extract. The investigator prescribed KaraCalm™ or a placebo as per the protocol-based randomization to the subjects.

The sponsor was responsible for the supply of all the investigational products (IPs), KaraCalm™ or placebo, in properly labelled packs and proper storage conditions. All the IPs were packed in white HDPE bottles. Three bottles were packed with 62 capsules for visit 3 (16 capsules), visit 6 (16 capsules), and visit 7 (30 capsules). The study medications were packed according to the assigned randomization number. Subjects were trained to take one capsule daily after dinner for 56 days and to document the consumption of IPs in a daily diary and compliance card. Any unused IPs were returned to the investigator or designated staff during the visits. Other nutritional supplements for sleep and stress were avoided during the participation in the study. Subjects, once enrolled, did not take any concomitant medications. Drugs that did not affect the clinical efficacy of KaraCalm™ may be allowed at the discretion of the principal investigator. Based on the investigators' opinion, rescue drugs (if any) were recorded in the source document and case report form. Subjects who required the use of rescue medication were considered a treatment failure.

***Physical examination and outcome measures***

Vital signs (heart rate, blood pressure, temperature, respiratory rate, and pulse rate) and weight were recorded at all visits. Each subject underwent the clinical laboratory tests, performed at screening and selected follow-up visits. Urine for a urinalysis and blood for hematology biochemistry were collected on screening and at the end of the study visit. For the hematology, biochemistry, and serology laboratory tests, blood samples were collected by direct vein-puncture of peripheral veins; approximately 5 to 8 ml of blood was obtained for clinical laboratory tests at screening visit (V1) and final visit (V9).

Circadian rhythm sleep was recorded using actigraphy for the entire study duration. For this purpose, an ActiWatch was provided to each subject. PSS scores were evaluated at Visits 1, 3, 6,7, and 9. Biomarkers such as cortisol levels and hs-CRP were evaluated at visits 3, 6, and 9. Study monitoring included a regular assessment of the number and type of adverse events (AEs) and serious adverse events (SAEs).

***Statistical analysis***

The final statistical analysis and results were depicted for 48 participants at the end of the study. Data was analyzed using IBM statistical package for the social sciences (SPSS) 21. For continuous variables, mean and SD were obtained, and for categorical variables, frequency and percentage. Analysis of variance (ANOVA) with post Hoc Tukey's was used for intragroup comparison.

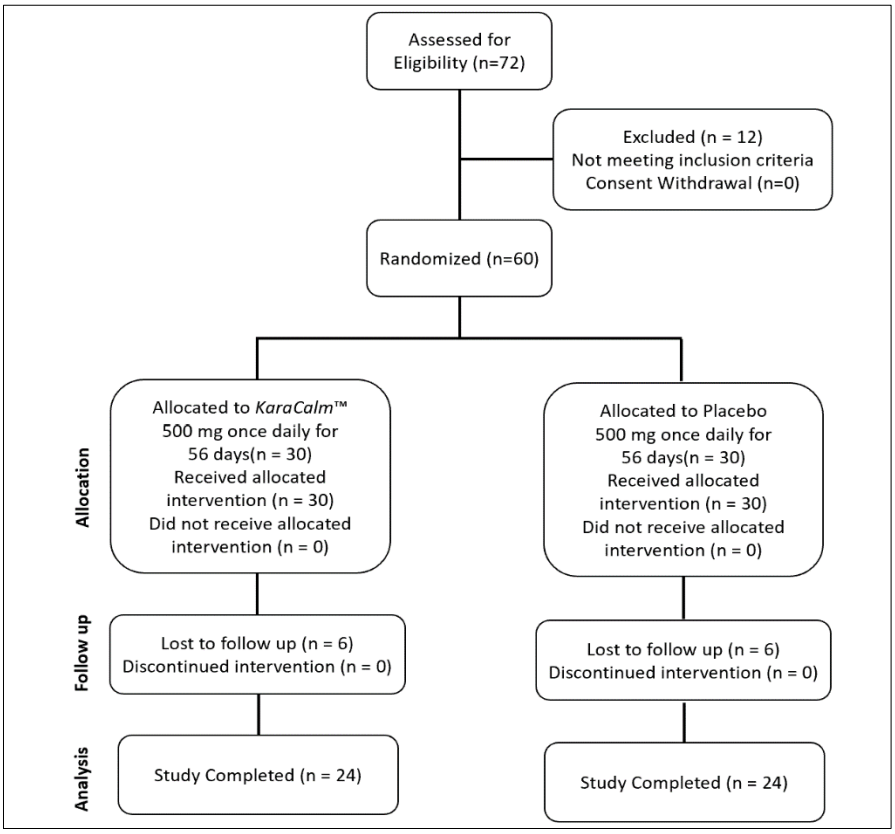


**Figure 1: Consort flow diagram of the study.**

## RESULTS

***Baseline characteristics***

Out of the 72 subjects screened for eligibility, 60 met the selection criteria and, following the signing of informed consent, were recruited into the study and randomized between KaraCalm™ (group A=30 subjects) or placebo (group B=30 subjects) group. Six subjects from group A and six from group B withdrew due to a loss of follow-up, resulting in 48 patients (24 subjects for each group). Demographic data did not differ statistically between groups; the mean age in group A was 31.38±9.59 years, and group B was 32.25±8.38 years. A summary of baseline demographic data of the subjects is given in Table 1.

***Sleep analysis***

Sleep quality analysis was performed using Actigraphy, a technique used to estimate sleep and wake patterns by measuring wrist movements with digital devices known as actigraphs; in the case of the present investigation, the ActiWatch was employed. Subjects in the KaraCalm™ group experienced a statistically significant increase in their get-up/waking up time compared to the Placebo group on day 14 and day 56, indicating that the

KaraCalm™ subjects had improved sleep and were not waking up earlier. The assessment of time in bed refers to the time spent in bed by a participant and is calculated by subtracting the time when the subject went to bed from the time when he/ she got up. This parameter showed that participants who were administered KaraCalm™ saw a statistically significant increase in the total time in bed on day 14 and day 56 compared to the placebo group, who experienced a decrease in their total bedtime. The total sleep time increased after the administration of KaraCalm™. Participants who were administered KaraCalm™ saw a statistically significant increase in their total sleep time (i.e. they slept for more time soundly with less time awake in bed) on day 14 and 56, compared to the placebo group participants. The placebo group saw a decrease in total sleep time from baseline to day 56 suggesting that participants were awake for more time and participants could not sleep efficiently.

**Table 1: Summary of subject's demography - PP analysis.**

| Variables | Group A (n=24) | Group B (n=24) |
|---|---|---|
| **Gender, N (%)** | | |
| Male | 11 (45.8) | 11 (45.8) |
| Female | 13 (54.2) | 13 (54.2) |
| **Age at baseline (N)** | 24 | 24 |
| Mean (SD) | 31.38 (9.59) | 32.25 (8.38) |
| (Min, max) | (21, 53) | (20, 53) |

The improvement of sleep quality in patients from the group treated with KaraCalm™ was further confirmed by evaluating the onset latency and sleep efficiency. Specifically, the evaluation of the onset latency showed that participants who were administered KaraCalm™ saw a statistically significant decrease in onset latency on day 14 and day 56 compared to the placebo group. KaraCalm™ subjects had a mean onset latency time of 17.5 minutes at baseline, and this decreased to 10.01 minutes on day 14 and 5.25 minutes on day 56. Participants who were administered KaraCalm™ experienced a statistically significant increase in sleep efficiency on day 14 and day 56 compared to the placebo group. Finally, the wake-after-sleep onset (WASO) minutes and number of awakenings were evaluated. Subjects in the KaraCalm™ group saw a statistically significant decrease in wake-up after sleep onset minutes on day 14 and day 56 compared to the placebo group, suggesting that after consumption of KaraCalm™, participants did not wake up as much in between during sleep and had a continuous sleep. In the same way, the KaraCalm™ group saw a statistically significant decrease compared to the placebo group on day 14 and day 56 in frequency of awakenings. Based on these results, it is possible to assert that KaraCalm™ represents a good natural supplement for improving sleep quality (Table 2).

***Evaluation of perceived stress***

In the present clinical trial, to evaluate the level of stress, the PSS was utilized. PSS is a widely used psychological instrument designed to measure the degree to which individuals perceive situations in their lives as stressful. It was formulated in 1983 and typically consists of a series of questions that assess the frequency of perceived stress over a defined period, such as the past month. Respondents indicate their level of agreement with each statement on a Likert-type scale, usually ranging from "never" to "very often." The PSS covers various aspects of stress perception, including feelings of unpredictability, lack of control, and coping ability. A statistically significant reduction in levels of perceived stress was observed in the KaraCalm™ group compared with placebo on day 14, day 28, and day 56 (Table 3).

***Evaluation of highly sensitive C-reactive protein levels***

Several investigations demonstrated that sleep disturbances and loss are related to alterations in metabolic function, immune system efficiency, and inflammatory markers, which might lead to the onset of cardiovascular diseases. Among the inflammation markers indicating an ongoing process of inflammation, the C-reactive protein (CRP) is one of the most used predictors of cardiovascular disease risk among the population. Specifically, the highly sensitive assay of CRP (hs-CRP) provides a more reliable microvascular rate of detection and was widely used for predicting risk for cardiac morbidity.[14] For these reasons, changes in hs-CRP were evaluated in the present study, demonstrating a gradual reduction in this biomarker in the KaraCalm™ group compared with placebo on day 14, day 28, and day 56 (Table 4).

***Evaluation of cortisol levels***

Sleep disorders are strongly correlated with elevated levels of cortisol as a result of HPA axis regulation impairment. This might lead to a glucocorticoid overload with a consequent higher risk of obesity and type II diabetes onset; for this reason, serum levels of cortisol were determined in this study, showing a reduction in the KaraCalm™ group compared with placebo on day 28 and day 56 (Table 5).[15]

***Safety evaluation***

The vital signs (systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse, respiration rate, and body temperature) were evaluated for all participants at every visit. Descriptive statistics were applied to all available data for safety evaluations; no significant changes were observed in the safety analyses of the patients in the intervention group when assessed from the start of the study to the end, thus indicating that KaraCalm™ was well tolerated and safe for consumption.

**Table 2: Comparison of sleep analysis actigraphy between KaraCalm™ (group A) and placebo (group B) at different visits.**

| Variables and groups | Mean | Standard deviation | Mean difference | P value |
|---|---|---|---|---|
| **Bed time (day 2)** | | | | |
| Group A | 20:59:39 | 4:31:25 | -0:55:56 | 0.328 |
| Group B | 21:55:35 | 0:55:15 | | |
| **Bed time (day 14)** | | | | |
| Group A | 21:34:39 | 1:07:18 | 0:30:38 | 0.601 |
| Group B | 21:04:01 | 4:37:00 | | |
| **Bed time (day 56)** | | | | |
| Group A | 21:35:39 | 4:09:03 | -0:44:00 | 0.015* |
| Group B | 22:19:39 | 1:03:02 | | |
| **Get up time (day 2)** | | | | |
| Group A | 5:22:08 | 0:48:38 | -0:12:00 | 0.406 |
| Group B | 5:34:19 | 0:51:48 | | |
| **Get up time (day 14)** | | | | |
| Group A | 6:06:46 | 0:58:04 | 0:54:05 | 0.000* |
| Group B | 5:12:41 | 0:38:56 | | |
| **Get up time (day 56)** | | | | |
| Group A | 6:54:57 | 1:24:36 | 2:12:21 | 0.000* |
| Group B | 4:42:35 | 1:02:04 | | |
| **Time in bed (day 2)** | | | | |
| Group A | 7:17:28 | 0:53:37 | -0:21:00 | 0.189 |
| Group B | 7:38:43 | 0:56:41 | | |
| **Time in bed (day 14)** | | | | |
| Group A | 8:26:25 | 1:51:20 | 0:59:40 | 0.019* |
| Group B | 7:26:45 | 0:44:12 | | |
| **Time in bed (day 56)** | | | | |
| Group A | 9:25:18 | 1:34:53 | 1:57:44 | 0.00* |
| Group B | 7:27:33 | 1:07:06 | | |
| **Total sleep time (day 2)** | | | | |
| Group A | 5:46:59 | 1:00:14 | -0:20:00 | 0.291 |
| Group B | 6:06:59 | 1:09:06 | | |
| **Total sleep time (day 14)** | | | | |
| Group A | 6:58:56 | 1:39:02 | 0:45:23 | 0.049* |
| Group B | 6:13:33 | 0:47:16 | | |
| **Total sleep time (day 56)** | | | | |
| Group A | 8:01:42 | 2:02:25 | 2:17:52 | 0.000* |
| Group B | 5:43:50 | 0:54:03 | | |
| **Onset latency day 2** | | | | |
| Group A | 17.51 | 6.70 | -0.23 | 0.913 |
| Group B | 17.73 | 7.51 | | |
| **Onset latency day 14** | | | | |
| Group A | 10.01 | 4.16 | -7.16 | 0.000* |
| Group B | 17.17 | 7.26 | | |
| **Onset latency day 56** | | | | |
| Group A | 5.25 | 3.17 | -12.39 | 0.000* |
| Group B | 17.64 | 10.04 | | |
| **Sleep efficiency day 2** | | | | |
| Group A | 77.31 | 4.94 | 1.80 | 0.363 |
| Group B | 75.51 | 8.20 | | |
| **Sleep efficiency day 14** | | | | |
| Group A | 81.85 | 6.31 | 5.50 | 0.004* |
| Group B | 76.35 | 6.42 | | |
| **Sleep efficiency day 56** | | | | |
| Group A | 86.58 | 4.54 | 9.02 | 0.000* |
| Group B | 77.56 | 6.54 | | |

Continued.

| Variables and groups | | Mean | Standard deviation | Mean difference | P value |
|---|---|---|---|---|---|
| **WASO - minutes day 2** | | | | | |
| Group A | | 104.92 | 22.32 | 47.35 | 0.000* |
| Group B | | 57.57 | 18.10 | | |
| **WASO - minutes day 14** | | | | | |
| Group A | | 75.63 | 14.31 | 17.95 | 0.001* |
| Group B | | 57.69 | 19.56 | | |
| **WASO - minutes day 56** | | | | | |
| Group A | | 60.72 | 16.63 | 9.42 | 0.041* |
| Group B | | 51.30 | 14.34 | | |
| **Awake (day 2)** | | | | | |
| Group A | | 26.00 | 4.95 | 0.65 | 0.683 |
| Group B | | 25.36 | 5.89 | | |
| **Awake (day 14)** | | | | | |
| Group A | | 18.55 | 5.68 | -6.33 | 0.000* |
| Group B | | 24.88 | 4.70 | | |
| **Awake (day 56)** | | | | | |
| Group A | | 12.32 | 5.32 | -12.12 | 0.000* |
| Group B | | 24.44 | 6.71 | | |
| **Pairwise comparison** | | | | | |
| | | | **Group A** | | **Group B** |
| **Dependent Variable** | **(I) Time interval** | **(J) Time interval** | **Mean difference (I-J)** | **P value** | **Mean difference (I-J)** | **P value** |
| **Bed time** | Day 2 | Day 14 | -0:34:59 | 0.743 | 0:51:34 | 0.536 |
| | | Day 56 | -0:35:59 | 0.731 | -0:24:03 | 0.872 |
| **Get up time** | Day 2 | Day 14 | -0:44:00 | 0.050* | 0:21:37 | 0.323 |
| | | Day 56 | -1:32:00 | 0.000* | 0:51:43 | 0.003* |
| **Time in bed** | Day 2 | Day 14 | -1:08:56 | 0.026* | 0:11:58 | 0.747 |
| | | Day 56 | -2:07:49 | 0.000* | 0:11:09 | 0.775 |
| **Total sleep time** | Day 2 | Day 14 | -1:11:57 | 0.033* | -0:06:33 | 0.918 |
| | | Day 56 | -2:14:43 | 0.000* | 0:23:09 | 0.350 |
| **Onset latency** | Day 2 | Day 14 | 7.50 | 0.000* | 0.56 | 0.971 |
| | | Day 56 | 12.25 | 0.000* | 0.10 | 0.999 |
| **Sleep efficiency** | Day 2 | Day 14 | -4.54 | 0.012* | -0.84 | 0.912 |
| | | Day 56 | -9.27 | 0.000* | -2.04 | 0.581 |
| **WASO-minutes** | Day 2 | Day 14 | 29.29 | 0.000* | -0.11 | 0.982 |
| | | Day 56 | 44.20 | 0.000* | 6.27 | 0.143 |
| **#AWAKE** | Day 2 | Day 14 | 7.46 | 0.000* | 0.48 | 0.956 |
| | | Day 56 | 13.68 | 0.000* | 0.92 | 0.848 |

*The mean difference is significant at p≤0.05

**Table 3: Comparison of PSS between KaraCalm™ (group A) and placebo (group B) at different time visits.**

| Variables and groups | Mean | Standard deviation | Mean difference | P value |
|---|---|---|---|---|
| **Day 2 PSS** | | | | |
| Group A | 21.58 | 1.32 | 0.08 | 0.830 |
| Group B | 21.50 | 1.35 | | |
| **Day 14 PSS** | | | | |
| Group A | 18.50 | 1.44 | -3.17 | 0.000* |
| Group B | 21.67 | 1.40 | | |
| **Day 28 PSS** | | | | |
| Group A | 15.71 | 1.88 | -5.75 | 0.000* |
| Group B | 21.46 | 1.56 | | |
| **Day 56 PSS** | | | | |
| Group A | 12.88 | 1.30 | -7.38 | 0.000* |
| Group B | 20.25 | 2.13 | | |

Continued.

| Pairwise comparison | | | Group A | | Group B | |
|---|---|---|---|---|---|---|
| **Dependent variable** | **(I) Time interval** | **(J) Time interval** | **Mean difference (I-J)** | **P value** | **Mean difference (I-J)** | **P value** |
| **PSS** | Day 2 | Day 14 | 3.08 | 0.000* | -0.17 | 1.000 |
| | | Day 28 | 5.88 | 0.000* | 0.04 | 1.000 |
| | | Day 56 | 8.71 | 0.000* | 1.25 | 0.059 |

*The mean difference is significant at p≤0.05

**Table 4: Comparison of hs-CRP KaraCalm™ (group A) and placebo (group B).**

| Variables and groups | Mean | Standard deviation | Mean difference | P value |
|---|---|---|---|---|
| **Day 2 hs CRP** | | | | |
| Group A | 2.53 | 0.67 | -0.15 | 0.428 |
| Group B | 2.68 | 0.62 | | |
| **Day 28 hs CRP** | | | | |
| Group A | 2.20 | 0.68 | -0.45 | 0.020* |
| Group B | 2.64 | 0.60 | | |
| **Day 56 hs CRP** | | | | |
| Group A | 0.91 | 0.11 | -1.68 | 0.000* |
| Group B | 2.60 | 0.59 | | |

| **Pairwise comparison** | | | | | | |
|---|---|---|---|---|---|---|
| **Dependent variable** | **(I) Time interval** | **(J) Time interval** | **Mean difference (I-J)** | **P value** | **Mean difference (I-J)** | **P value** |
| **Hs-CRP** | Day 1 | Day 28 | 0.34 | 0.102 | 0.04 | 0.976 |
| | | Day 56 | 1.62 | 0.000* | 0.08 | 0.882 |

*The mean difference is significant at p≤0.05

**Table 5: Comparison of cortisol serum levels KaraCalm™ (group A) and placebo (group B).**

| Variables and groups | Mean | Standard deviation | Mean difference | P value |
|---|---|---|---|---|
| **Day 2 serum cortisol** | | | | |
| Group A | 16.85 | 2.21 | 0.60 | 0.312 |
| Group B | 16.26 | 1.82 | | |
| **Day 28 serum cortisol** | | | | |
| Group A | 13.61 | 0.92 | -2.37 | 0.000* |
| Group B | 15.98 | 1.05 | | |
| **Day 56 serum cortisol** | | | | |
| Group A | 11.72 | 1.19 | -4.23 | 0.000* |
| Group B | 15.96 | 1.28 | | |

| **Pairwise comparison** | | | | | | |
|---|---|---|---|---|---|---|
| **Dependent variable** | **(I) Time interval** | **(J) Time interval** | **Mean difference (I-J)** | **P value** | **Mean difference (I-J)** | **P value** |
| **Serum Cortisol** | Day 2 | Day 28 | 3.25 | 0.000* | 0.28 | 0.776 |
| | | Day 56 | 5.13 | 0.000* | 0.30 | 0.744 |

*The mean difference is significant at p≤0.05

## DISCUSSION

Stress is an inevitable aspect of daily life and is linked with significant health challenges, including increased morbidity and mortality. The increase in generalized anxiety and sleep disturbance seen with chronic stress lead to notable physiological issues such as cardiovascular, gastrointestinal, and immunological problems. Alongside lifestyle adjustments such as maintaining a healthy diet, exercising, and practicing meditation, several nutrients and herbal remedies can support stress-related conditions. From this background, a novel herbal formulation was born, KaraCalm™, which includes 6 different herbal extracts known for reducing stress and improving sleep quality.

Therefore, the present randomized, double-blind, parallel, placebo-control study was designed to evaluate the safety and efficacy of KaraCalm™ to assess its properties for managing stress and improving sleep among adults. Healthy adults, male and female subjects aged 18 to 54 years, free of psychiatric conditions other than mild stress and willing to provide written informed consent, were enrolled in the study. Using the actigraphy technique, a

significant increase in sleep quality was observed in the treatment group on day 14 and day 56, which indicated more restorative and peaceful sleep compared to the placebo group. Improved sleep quality has been proven to enhance health, reduce daytime sleepiness, and promote psychological functioning and well-being. Sleep latency refers to the duration of a subject falling asleep after switching off the lights. Comparison between groups on the 14th and 56th days showed a significant reduction in latency scores, indicating the positive impact of KaraCalm™ compared to the placebo. This improvement in the treated group was also observed for other variables related to sleep as well such as total bed time, sleep efficiency, wake after sleep onset minutes, and number of awakenings once subject has fallen asleep, thus confirming the effectiveness of KaraCalm™.

KaraCalm™ not only improved sleep quality but also reduced the subjects' stress levels assessed using the PSS, in which a high score indicates a higher stress perception. It provides a degree measure of the extent to which a person's life is influenced by stress and anxiety.[16] Previous clinical trials supported the observed effectiveness of KaraCalm™ since all its constituents, *Valeriana officinalis* extract, *Passiflora incarnata* extract, *Ocimum sanctum* extract, *Ziziphus jujuba* extract, *Rosmarinus officinalis* extract, and *Nigella sativa* extract, showed the ability in reducing stress and improving sleep quality in clinical trials.[1,12,13,16,17]

Generally, it is known that cortisol secretion is strongly rhythmic and remains at a low level in the day and early night but increases during the latter part of the night towards the morning. Cortisol nadir occurs within 2 hours of sleep onset; this means that sleep initiates when cortisol is lowest and terminates when cortisol is highest. Physiological or physical stress modifies cortisol secretion by triggering the HPA axis, disrupting the typical circadian pattern of cortisol release.[2] A study involving military cadets exposed to a rigorous five-day training regimen involving intense physical activity, as well as food and sleep deprivation, observed an elevation in cortisol levels and a decline in performance due to the stress-inducing nature of the training. Researchers noted that the cadets' circadian rhythm was disrupted, and even after 4-5 days of rest, their circadian rhythms had not fully returned to normal.[18] This evidence was also supported in another clinical trial on healthy adults in which acute sleep deprivation is linked to increased cholesterol levels and a general inflammatory state.[19] KaraCalm™ supplementation decreases cortisol serum levels on day 28 and day 56, thus confirming the reduction in stress levels assessed with PSS. The regulation of serum cortisol concentration might be attributed to the presence in the formulation of *Rosmarinus officinalis* extract and *Nigella sativa* extract, as previous clinical investigations have demonstrated a decrease in this stress marker.[16,20]

Emerging new evidence suggests that sleep may be strongly implicated in cardio-metabolic human health by regulating inflammatory pathways. Previous research has demonstrated a strong connection between sleep deprivation and increased inflammatory biomarkers such as tumour necrosis factor $\alpha$ (TNF-$\alpha$), interleukin-6 (IL-6), and acute-phase reactant CRP. In addition, other investigations suggested that either self-reported sleep disturbance or poor sleep quality was associated with an inflammatory state. Within the several inflammatory biomarkers indicating inflammatory responses, CRP is one of the most frequently employed predictors of cardiovascular disease risk.[14] A gradual reduction over time in hs-CRP levels was demonstrated in the present clinical study in the KaraCalm™ group compared with placebo on day 28 and day 56, evidencing a decrease in the inflammatory state related to stress and sleep disturbances. It is also possible to hypothesize a reduction in other inflammatory markers and increased immune system activity since another natural supplement containing the extract of *Ocimum sanctum* L., KaraShield™, demonstrated immunostimulating and anti-inflammatory activities.[21]

The absence of relevant adverse events, side effects, or toxic manifestations in clinical laboratory measurements (biochemical/haematological) suggested the suitability and safety of KaraCalm™. However, the small sample size of the investigated patients could be viewed as a limitation of the current study.

## CONCLUSION

The present randomized, double-blind, parallel, placebo-control study of 56 days was designed to evaluate the safety and efficacy of KaraCalm™ to assess its properties for managing stress and improving sleep among adults. For the KaraCalm™ group, there was an increase in total sleep time and sleep efficiency and a decrease in sleep onset latency, WASO minutes, and frequency of awakenings, indicating the supplement's effectiveness in improving sleep quality. KaraCalm™ patients also saw a decrease in PSS scores in the treated group from baseline to the end of the study, indicating improved sleep quality and reduced stress levels, respectively. A significant reduction in serum cortisol and hs-CRP levels in the KaraCalm™ group from baseline to the end of the study further supported the effectiveness of KaraCalm™ in reducing stress. Finally, there was no significant change in the safety analyses of the patients in the intervention group when assessed from the start of the study to the end, thus indicating that KaraCalm™ was well tolerated and safe for consumption. Therefore, based on the overall results, it appears that KaraCalm™ is a safe and effective dietary herbal supplement to reduce stress and improve sleep quality.

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## ANNEXURE

### Events schedule chart.

| Visits* | Visit 1 Day -3 | Visit 2 Day -1 /Day 0 | Visit 3 Day 0/ Day 1 | Visit 4 Day 3/ Day 4 | Visit 5 Day 9/ Day 10 | Visit 6 Day 13 / Day 14 | Visit 7 Day 28 | Visit 8 Day 51 /Day 52 | Visit 9 Day 55 /Day 56 |
|---|---|---|---|---|---|---|---|---|---|
| **Informed consent** | X | - | - | - | - | - | - | - | - |
| **Eligibility criteria** | X | - | - | - | - | - | - | - | - |
| **Demographic data** | X | - | - | - | - | - | - | - | - |
| **Medical history** | X | - | - | - | - | - | - | - | - |
| **Physical examination** | X | - | X | - | - | X | X | - | X |
| **Vital signs** | X | - | X | - | - | X | X | - | X |
| **Concomitant medications** | | - | X | - | - | X | X | - | X |
| **Safety-laboratory investigations-CBC, LFT, RFT, ECG, Lipid profile** | X | - | - | - | - | - | - | - | X |
| **HIV, RBS and UPT** | X | - | | - | - | - | - | - | - |
| **Bio markers Cortisol, hsCRP 3 times** | - | - | X | - | - | X | - | - | X |
| **Anti-stress scales #** | X | - | X | - | - | X | X | - | X |
| **Randomization** | - | - | X | - | - | | | - | |
| ***IP reconciliation/ dispensing** | - | - | X | - | - | X | X | - | X |
| **Adverse Events** | - | - | - | - | - | X | X | - | X |
| **End of treatment** | - | - | - | - | - | | | - | X |
| **End of study** | - | - | - | - | - | - | - | - | X |
| **Actigraphy in 60 subjects each – 3 times** | X | X | X | X | X | X | - | X | X |

* Each visit had a window period: +/-3 days