

Mood and Sleep Benefits of Mushroom Supplementation in Young Adults: An Exploratory Randomized, Double-Blind, Placebo-Controlled Trial

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ABSTRACT

Mood disturbances and poor sleep are increasingly prevalent in young adults, fueling interest in natural alternatives.

Background: This exploratory randomized, double-blind, placebo-controlled, four-arm parallel-group trial evaluated the effects of an organic mushroom extract blend on mood, sleep quality and salivary cortisol.

Methods: Eighty adults (aged 18-40) with self-reported poor sleep and mood symptoms were randomized (1:1:1:1) to receive capsules or snack bars, with or without 250 mg mushroom blend, divided into equal servings and taken twice daily for 25 days. Primary outcomes were changes in Depression Anxiety Stress Scales-21 (DASS-21) and Pittsburgh Sleep Quality Index (PSQI) scores; secondary outcomes included salivary cortisol. Safety was assessed *via* vital signs and hematological markers, with analyses conducted using nonparametric statistics. Trial registration: Clinical Trials Registry-India (CTRI/2024/02/063100).

Results: Seventy-eight participants completed the trial. Both mushroom groups showed significant reductions in DASS-21 and PSQI scores at Days 13 and 25 compared with placebo ($p < 0.05$), exceeding any changes in placebo groups. Salivary cortisol was also significantly reduced at Day 25 in mushroom groups *versus* placebo ($p < 0.001$). No treatment-related adverse events or clinically significant changes in safety markers were observed.

Conclusions: Supplementation with an organic mushroom extract blend improved mood, sleep quality and physiological stress markers, with no safety concerns. These findings suggest benefits may be mediated, at least in part, through modulation of the Hypothalamic Pituitary Adrenal (HPA) axis. Both capsule and snack bar formats were effective, safe and well tolerated, supporting the blend's potential as a natural strategy for stress and sleep support.

Keywords: Mushroom bioactives; Sleep quality; Mood; Cortisol; Adaptogens; Young adults; Functional foods; Hypothalamic Pituitary Adrenal (HPA)

Abbreviations: MB: Mushroom Blend; PLA: Placebo; CAP: Capsule; BAR: Nutritional Snack Bar; HPA: Hypothalamic Pituitary Adrenal; DASS-21: Depression, Anxiety and Stress Scales; PSQI: Pittsburgh Sleep Quality Index; ANCOVA: Analysis of Covariance; CRO: Contract Research Organization

INTRODUCTION

There are emerging interests and research investigating the relationship between daily stressors, mood, anxiety and sleep quality. Poor mood and anxiety are often considered independently, however, a systematic review determined robust and consistent evidence of comorbidity between broadly defined mood and anxiety disorders [1]. Epidemiological studies show that sleep disturbances, particularly insomnia, affect roughly half of those coping with anxiety and that poor sleep can function to initiate

or further exacerbate it [2]. While interest extends throughout adulthood, younger adults are experiencing anxiety and mood issues more significantly than previous generations. A recent study looked at the prevalence of anxiety and/or depression symptoms in 2,809 young adults aged 18-25 years. Nearly 50% of young adults experienced mental health symptoms during a global pandemic. Of those experiencing symptoms, 39% had reported using prescription medications and/or had received mental health support, while 36% reported they were unable to access mental health counseling [3]. Reduced availability to mental health support services and/

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or medications, the search for more natural, plant-based solutions continues to gain consumer interest.

Improved sleep and mood may enhance emotional regulation and stress resilience, potentially facilitating the development of intimacy and the successful navigation of this psychosocial stage [4]. Young adults who participated in nine years of longitudinal sleep research showed significantly reduced sleep quantity and quality [5]. The relationship between young adults' usage of social media and sleep disruptions was also examined in a study involving 1788 US young adults between the ages of 19 and 32. According to research data extrapolated, there was a significantly larger likelihood of suffering sleep problems, such as trouble falling asleep, staying asleep, or experiencing restless sleep, while using social media more frequently and in greater volume [5]. There is a recognized need for healthier sleep solutions in this age group, along with growing interest in plant-based remedies to help improve both sleep quantity and quality.

Cortisol plays a central role in the body's stress response and has been widely studied as a biomarker linking psychological and physiological health. Cortisol is a stress marker for the body and levels typically increase in response to physical or psychological stress. Poor mood and anxiety are often associated with dysregulation of cortisol. More specifically, hypercortisolemia may be attributed to a dysfunction of glucocorticoid-receptor-mediated negative feedback mechanisms within the Hypothalamic Pituitary Adrenal (HPA) axis, which in turn can play a pivotal role in mood disorders, characterized by depressive symptoms and cognitive deficits [6]. Higher salivary cortisol levels can indicate elevated stress and irregular cortisol patterns reflecting possible disruptions in circadian rhythms and/or sleep issues.

Individual or blended mushroom components, extracts and concentrates have been used in traditional medicines and applied to address conditions related to cognition, mood and sleep [7-10]. Different mushroom species vary in the presence and concentrations of bioactive compounds that can directly or indirectly affect cognition, mood and sleep, including vitamins, beta glucans (β -glucans), terpenoids, diterpenoids, polyphenols and sterols [7,10]. Mushroom polysaccharides are of differing chemical composition, the majority being beta-glucans; these have beta linkages (1 to >3) in the primary chain of glucan and subsequent beta branch points (1 to >6) which are required for their biological action [11].

Some mushrooms, typically phenolics or tetracyclic triterpenoids/steroids, are considered adaptogens, purported to support managing typical life stressors [8,9]. Adaptogens are naturally occurring bioactive compounds found in whole mushrooms and mushroom extracts consisting usually of either complex plant phenolics or tetracyclic triterpenoids/steroids classified by their relation to a physiological process, notably adaptation to environmental challenges that support resistance to life stressors by modulating or regulating the cortisol response [7-9].

For instance, *Ganoderma lucidum* is a fungus commonly known as Lingzhi or Reishi and is reported to have analgesic and sedative potential as well as reduce fatigue and states of sadness, which in turn can affect mood [12,13]. In Traditional Chinese Medicine (TCM), Reishi mushroom is esteemed for its ability to soothe the mind and promote relaxation, effects attributed to its adaptogenic properties and the presence of triterpenes [14]. Reishi is also traditionally recognized for its ability to calm the mind and restore balance

to the body [15]. Its adaptogenic activity is attributed to a diverse array of bioactive compounds, notably triterpenes, specifically ganoderic acids, which exhibit a molecular structure similar to steroid hormones [14]. Reishi also contains biologically active polysaccharides and over 200 other constituents, many of which are among the most pharmacologically active identified in natural sources [14,16]. Meanwhile, components of *Cordyceps militaris* have anti-inflammatory and antioxidant properties and its application is associated with mood [17]. Moreover, the *Cordyceps* genus, inclusive of species *militaris* and *sinesis*, are characterized by numerous bioactive compounds including unique cyclodepsipeptides, nucleosides and polysaccharides which may play a role in supporting cognition and more desirable mood states [18-20]. These effects have also been attributed to the presence of cordycepin, adenosine, ergosterol and multiple amino acids [21]. At the same time, there is growing interest in *Hericium erinaceus* (Lion's Mane) for its potential neuroprotective and neuro-regenerative properties [22,23]. Lion's Mane shows promise for more immediate application related to mood, stress and states of worry improvement [24]. It has been increasingly studied for the potential to support cognitive function, neurological health and mood regulation [25]. Supplementation of Lion's Mane was reported to improve some measures of cognitive function, perceived stress and mood in young adults (ages 18-45) [26].

Delivery form is another important aspect of dietary supplementation when considering regulatory approval, ingredient interactions, bioactive stability, bioavailability, etc. Traditionally, botanical-based dietary supplements have mostly been marketed in pill form (e.g. capsules, tablets, soft gels, etc.) and secondarily ready-to-mix powders. Variety and lifestyle application is a key consideration and many people look for alternative delivery forms of dietary supplementation. For instance, mushroom ingredients have been explored as an option for coffee [27]. Meanwhile, functional bar formats can offer advantages related to delivering additional nutrients as well as sensory properties that may overcome flavor masking challenges associated with many botanical ingredients including mushrooms [28,29].

There is widespread anxiety, disturbed mood and sleep disruption in young adults. There is an ongoing quest for more natural remedies for these conditions. Considering these aspects, the primary purposes of this investigation were to evaluate the efficacy of a commercially available proprietary organic, standardized mushroom blend, either as capsules or part of a functional bar on mood, sleep quality and duration and salivary cortisol levels. We hypothesized that 25-day supplementation with MB would improve mood states of depression, anxiety and stress compared to placebo in young adults as a result of secondary improvements expected in sleep quality and salivary cortisol levels.

MATERIALS AND METHODS

This randomized, double-blind, placebo-controlled trial was conducted by BioAgile Therapeutics Pvt. Ltd. at Sri B.M. Patil Medical College Hospital in Vijayapura, Karnataka, India. The study was designed to evaluate the effects of an organic mushroom extract blend of *Ganoderma lucidum* (Reishi mushroom), *Cordyceps militaris*, *Hericium erinaceus* (Lion's Mane) and fermented *Cordyceps sinensis* on mood, sleep quality and salivary cortisol levels in healthy young adults. The protocol received approval on January 29, 2024, from the Sri B.M. Patil Medical College Hospital's Institutional Ethics Committee. The trial was registered with the Clinical Trials

Registry-India in February of 2024 (CTRI/2024/02/063100). Participants and the public were not involved in the design, conduct, reporting, or dissemination of this research.

Participants

A total of 80 men and women aged 18-40 were enrolled and randomly assigned to one of four groups (n=20 per group) to mitigate systematic bias: Placebo Capsule (PLA-CAP), placebo functional snack bar (PLA-BAR), organic mushroom extract blend capsule (MB-CAP), or organic mushroom extract blend functional bar (MB-BAR). Each group received their assigned intervention

twice daily, once in the morning (with or without food) and once in the evening (with or without food), for a period of 25 days. The experimental design and treatment allocation are shown in Figure 1 and demographic characteristics of the study population are presented in Table 1. Assessments were performed at baseline (Day 0), Day 13 and at study completion (Day 25), focusing primarily on the Depression, Anxiety and Stress Scales-21 psychological questionnaire (DASS-21) and validated with the Pittsburgh Sleep Quality Index (PSQI) questionnaire and salivary cortisol analysis. Other outcomes included vital signs and hematological and liver integrity analysis for safety parameter assessment.

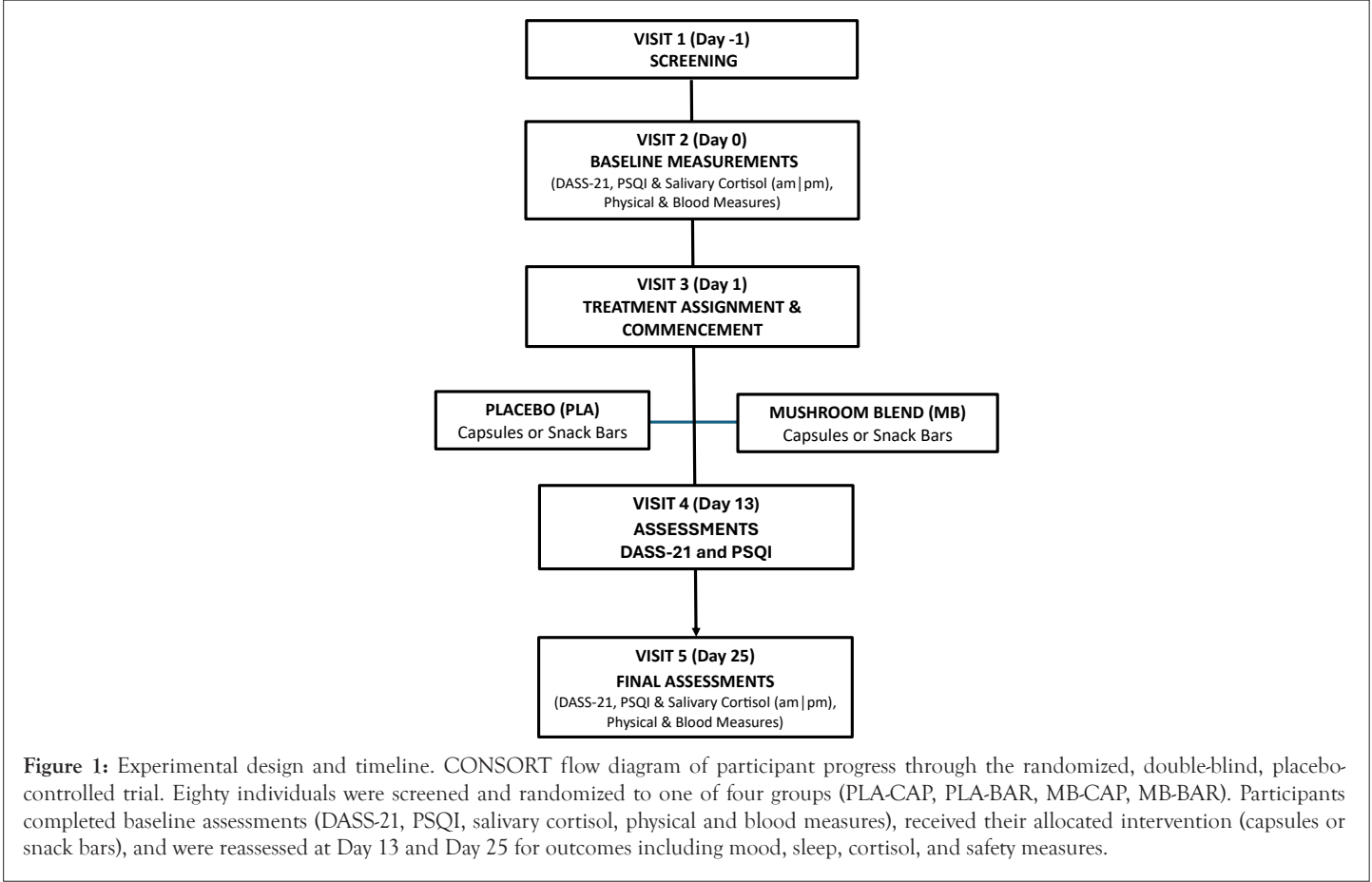


Table 1: Gender, age and anthropometrics.

	PLA-CAP	MB-CAP	PLA-BAR	MB-BAR
Gender (female/male)	11 9	7 13	9 11	14 6
Age (years)	28.10 ± 6.67	28.05 ± 5.89	28.25 ± 5.51	27.10 ± 5.79
Height (cm)	159.7 ± 3.85	162.9 ± 4.09	160.8 ± 6.12	159.9 ± 3.64
Weight (kg)	59.55 ± 11.27	62.95 ± 11.61	61.50 ± 8.45	61.68 ± 8.45

Note: Mean ± SD; PLA-CAP:Placebo Capsule | PLA-BAR:Placebo Bar
MB-CAP:Mushroom Blend Capsule | MB-BAR:Mushroom Blend Bar

Inclusion criteria

Inclusion criteria included experiencing feelings of fatigue, weakness and/or tiredness, moodiness, irritability and/or restlessness. Additional inclusion criteria comprise chronic lack of concentration, fear and/or worry, difficulty completing tasks and/or lack of motivation, loneliness and/or withdrawal from or lack of interest in social groups. Inclusion criteria for sleep disturbances included changes in sleeping patterns or habits and chronic difficulty falling or staying asleep, daily grogginess.

Exclusion criteria

Individuals were excluded from participation if they had been using antidepressant medications, such as Selective Serotonin Reuptake Inhibitors (SSRIs) or Monoamine Oxidase Inhibitors (MAOIs), or any central nervous system depressants, including benzodiazepines, barbiturates, beta blockers, opioids, or recreational substances like cannabis. Use of tobacco products, including vaping devices, also disqualified participants. Individuals with diagnosed diabetes or pre-diabetic conditions were excluded due to potential metabolic variability and risk of glycemic instability. Additional exclusions applied to those who were pregnant or breastfeeding, or who had known allergies to mushrooms or mushroom-derived ingredients. No concomitant treatments or supplements affecting mood, sleep, or cortisol were permitted during the trial and participants were instructed to maintain their usual diet and activity levels. Primary emphasis was placed on ruling out participants whose current medications, metabolic status, psychiatric history, or physiological state could interfere with the study outcomes or pose safety concerns.

Final eligibility was confirmed by the Principal Investigator.

Dietary ingredient supplementation

This was a parallel-group, four-arm clinical trial in which participants were randomly assigned in a 1:1:1:1 ratio using block randomization. The allocation sequence was implemented using sealed opaque envelopes prepared by the Contract Research Organization (CRO) in consultation with a statistician and envelopes were opened at Visit 3 (Day 1) to assign participants. To maintain double-blind conditions, active and placebo capsules were matched in size, shape, color and texture and were dispensed in sealed bottles that were identical in appearance and labeling. Similarly, active and placebo chewable snack bars were made using the same base formulation and individually wrapped in visually identical packaging.

Participants received either a capsule or a functional snack bar, which was to be consumed twice daily (morning and early evening). Capsules were vegetarian compatible (Hydroxypropyl Methylcellulose (HPMC)) utilizing maltodextrin as an excipient. Participants in CAP groups consumed one capsule twice daily. Snack bars (BAR) were prepared with/without MB and were 25 grams per bar consumed twice daily. See Table 2 for composition details for both capsules and bars. Participants were instructed to avoid initiating any new medications, supplements, or treatments during the trial period. No concomitant care was reported during the study. Compliance was tracked through a combination of returned product counts and participant-reported use *via* a designated field worker. According to study records, all products were taken as directed and no deviations from the protocol were noted.

Table 2: Placebo and active compositions.

	PLA-CAP	MB-CAP	PLA-BAR	MB-BAR
Serving Size	300 mg	300 mg	25 g	25 g
Energy (calories)	0	0	85	85
Protein (g)	0	0	2	2
Fat (g)	0	0	1.5	1.5
Carbohydrates (g)	0	0	17	17
Total Sugar (g)	0	0	7.5	7.5
Flavor	unflavored	unflavored	vanilla	vanilla
Active Ingredient (%)	0% (0 mg)	41.7% (125 mg)	0% (0 mg)	0.5% (125 mg)
Other Ingredient(s)	100% (230 mg) tapioca maltodextrin	58% (175 mg) tapioca maltodextrin	Date paste, rolled oats (gluten free), tapioca syrup, rice crisps (rice flour, sugar, sea salt), pea protein, vegetable glycerin, sunflower oil, natural flavor, salt, sunflower lecithin	Date paste, rolled oats (gluten free), tapioca syrup, rice crisps (rice flour, sugar, sea salt), pea protein, vegetable glycerin, sunflower oil, natural flavor, salt, sunflower lecithin, cocoa

Note: PLA-CAP-Placebo Capsule | PLA-BAR Bar
MB-CAP-Mushroom Blend Capsule | MB-BAR Mushroom Blend Bar

Organic mushroom blend

Organic mushroom extract blend (distributed by NURA USA, ADAPTGUARD®) consists of organic *Ganoderma lucidum* (Reishi) fruiting body extract, organic *Cordyceps militaris* fruiting body extract, organic *Hericium erinaceus* (Lion's Mane) fruiting body extract and fermented *Cordyceps sinensis* mycelium extract. This is a full-spectrum blend with a minimum of 28% beta glucans. The total daily intake of the mushroom extract blend was 250 milligrams, split between morning and evening supplementation of 125 milligrams each time and provided as a snack bar or in capsule form. Additional documentation regarding the mushroom extract blend, including the manufacturer's specification sheet and certificate of analysis, is available upon reasonable request from the corresponding author.

Allocation concealment

Participants and scientists were blinded to treatments until completion of the study. Allocation concealment was ensured through secure storage of randomization codes in tamper-evident envelopes and a master randomization chart was sealed and stored in both physical and secure electronic formats. Unblinding was permitted only under specific conditions, such as medical emergencies or serious adverse events and could only be performed by authorized personnel not involved in the day-to-day management of the trial.

Assessments

DASS-21The Depression, Anxiety and Stress Scales-21 items (DASS-21) report is a psychological questionnaire that was used to measure the dimensions of depression, anxiety and stress [30]. The DASS-21 psychological questionnaire was administered at the testing site at baseline (Day 0), Day 13 and final assessment (Day 25).

PSQIThe Pittsburgh Sleep Quality Index (PSQI) questionnaire is commonly used in both clinical and research settings to assess several aspects of sleep quantity and quality, including sleep duration, sleep disturbances and overall sleep satisfaction [31]. The PSQI was surveyed at the test site at baseline (Day 0), Day 13 and final assessment (Day 25).

Salivary cortisolSalivary cortisol was tested in the morning and evening at baseline (Day 0) and final assessment (Day 25). Saliva samples were collected in test tubes at the testing site by the authorized field worker, stored accordingly and analyzed on site. Cortisol is often measured as an indicator of mood states of sadness, anxiousness and other stress-related conditions during clinical research.

Physical assessments

Individual height and weight were measured by standing stadiometer and balance scale. Oral temperature was assessed as average of three measures per participant *via* calibrated thermometer and average measure was used. The heart rate, as beats per minute (bpm), was measured three times by radial artery, each separated by one minute of comfortable rest in a cushion-bottom, back supported chair with feet flat and legs uncrossed on an adjustable height platform and without pressure under knee joint. Systolic and diastolic blood pressures were assessed in triplicate with 1-minute rested intervals immediately after heart rate assessment set utilizing an automated upper-arm pressure cuff.

Hematology assessment

Following physical assessments, blood was captured *via* antecubital fossa vein or median cephalic and median basilic veins per professional assessment. Samples were assessed for red and white blood cell measures including Basophils (%), Eosinophils (%), Hemoglobin (gram/deciliter), Lymphocytes (%), Mean Corpuscular Hemoglobin (Pg), Mean Corpuscular Hemoglobin Concentration (Pg), Mean Corpuscular Volume (fL), Monocytes (%), Neutrophils (%), PCV (%), Platelet Count (Lakhs/cubic mm), Red Blood Cell (RBC) Count (millions/cubic mm) and White Blood Cell (WBC) Count (millions/cubic mm) at baseline (Day 0) and final assessment (Day 25).

Liver integrity and function assessment

Addition to hematology measures above, liver enzyme levels tests, namely Alanine Transaminase (ALT/SGPT) (IU/I) and Aspartate Aminotransferase (AST/SGOT) (IU/I), as well as Serum creatinine (mg/dl) were determined at baseline (Day 0) and final assessment (Day 25).

Statistical assessment

Data entry and statistics were completed using Microsoft Excel and GraphPad Prism 10. Normality was tested using D'Agostin and Pearson (K2) and Shapiro Wilk (W) tests. Based on that outcome, the Wilcoxon signed-rank test was applied to compare baseline values with follow-up (Day 13 and Day 25) within each group for DASS-21 scores, PSQI scores and salivary cortisol. The alpha priori was set at 0.05 ($p < 0.05$). The efficacy evaluation included all participants who were randomized and completed at least one assessment following baseline. Participants were evaluated within the groups they were originally randomized to, preserving the structure of the trial design. The dataset used for final analysis included only the data collected. No values were estimated or imputed. The statistical approach was defined in advance in the study protocol and centered on assessing changes in mood, sleep quality and salivary cortisol. A total sample size of 80 participants ($n=20$ per group) was selected based on feasibility for a pilot randomized controlled trial and consistency with sample sizes used in similar investigative studies assessing mood, sleep and stress outcomes. This size was considered adequate to detect a moderate effect and to estimate variability for future confirmatory trials. For this study, no additional subgroup or sensitivity analyses were conducted, as they were not pre-specified in the registered protocol, the sample size was not powered for such analyses and performing them could have increased the risk of Type I error and generated potentially misleading results. No interim analyses or stopping guidelines were planned or implemented for this study, as it was a short-term pilot trial without predefined criteria for early stopping. Two participants (one from GS-BAR and one from PLA-BAR) withdrew before study completion due to personal reasons and were excluded from the final Day 25 analysis. No adverse events were reported.

RESULTS

DASS-21 Score (Mood)

Table 3 and Figure 2 presents scores and changes in DASS-21 scores from baseline (Day 0), Day 13 and final assessment (Day 25) of treatment. Participants were randomized to treatment arms

to minimize bias and distribute potential confounding variables. Despite randomization, some baseline differences in DASS-21 scores were observed between groups, which can occur by chance, particularly in modestly sized samples. Significant improvements ($p<0.05$) in mood states of sadness, anxiousness and stress were determined between baseline survey (Day 0), Day 13 and final assessment (Day 25) for both MB treatment groups. The scores and changes in DASS-21 scores with the organic mushroom extract treatment in both delivery forms were different from both PLA treatments. All treatments were observed to change from baseline (Day 0) to final assessment (Day 25) with the changes of the MB treatment groups being significantly greater than the changes for the PLA treatments in both applications.

Sleep quality-PSQI

Despite random assignment baseline PSQI scores differed across treatment groups, with notably higher scores in the MB-CAP and MB-BAR groups. These differences are likely attributable to random variation, which can occur in modestly sized samples. To account for this, change-from-baseline analyses were performed and presented. There was a significant change in PSQI Score from baseline to post-baseline visits as presented in Table 4 and Figure 3. Higher PSQI scores indicate more severe sleep problems. Lower PSQI scores indicate improvements in sleep quality. Participants experienced a statistically significant improvement in overall sleep quality as results showed by reducing PSQI scores recorded with either MB-CAP and MB-BAR treatments consumed *versus* PLA of the same forms at Day 13 and final assessment (Day 25). All treatments showed changes from baseline (Day 0) to the final assessment (Day 25); however, the changes observed in the MB treatment groups were significantly greater than those in the PLA

groups across both applications.

Salivary cortisol

As presented in Table 5 and Figure 4, no significant differences were determined between morning and evening measures on Day 0 for any of the groups. Meanwhile, significant reductions ($p<0.001$) in salivary cortisol were determined between baseline (Day 0) and final assessment (Day 25) for both PLA and MB treatments of both forms at the time of day. MB treatment groups were significantly greater in change in salivary cortisol than the changes for the PLA treatments in both applications. These changes aligned with improvements in mood as assessed by DASS-21 on final assessment (Day 25). Although participants were randomized to treatment groups, baseline salivary cortisol levels varied across conditions. The MB-CAP group exhibited higher baseline cortisol levels than other arms, which may reflect random variation due to sample size and individual variability in physiological stress responses. Such differences are not uncommon in randomized trials, especially when sample sizes are modest.

Oral temperature, heart rate and blood pressure measures

The results of baseline (Day 0) and final assessments (Day 25) for oral temperature, heart rate, blood pressures are presented in Table 6. There were no significant differences that were determined in the change in oral temperature, heart rate and blood pressure measures from baseline (Day 0) to final assessments (Day 25) and are considered supportive of short-term tolerance and general safety.

Hematology, serum enzymes and creatinine

Table 3: DAAS-21 Time course scores.

	PLA-CAP	MB-CAP	PLA-BAR	MB-BAR
DAY 0	27.80 $\pm$ 6.51 n=20	34.80 $\pm$ 7.98 n=20	24.80 $\pm$ 4.15 n=20	27.15 $\pm$ 6.54 n=20
DAY 13	26.80 $\pm$ 6.51 n=20	29.70 $\pm$ 7.39* n=20	24.00 $\pm$ 4.26 n=19	23.82 $\pm$ 6.66* n=17
Day 25	25.45 $\pm$ 6.57* n=20	25.25 $\pm$ 6.26* n=20	23.22 $\pm$ 4.18* n=18	20.06 $\pm$ 5.62* n=17

Note: Presents DASS-21 scores (Mean $\pm$ SD) from baseline at Day 13 and 25. *Indicates significant changes ($p<0.05$) in DASS-21 scores on Day 13 and 25 as compared to Baseline (Day 0). PLA=Placebo; MB=Mushroom Blend; CAP=Capsules; BAR=Nutrition Bar

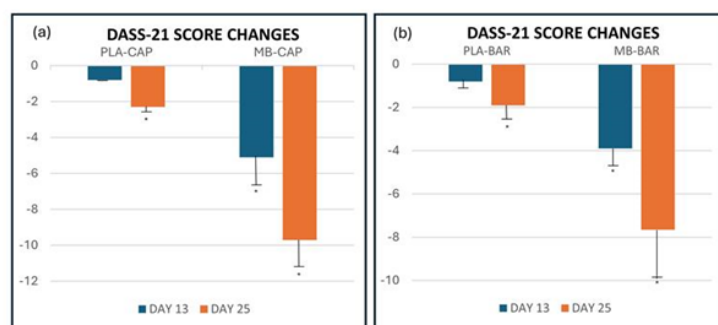


Figure 2: Presents changes in DASS-21 scores (Mean $\pm$ SD) from baseline (Day 0) at Day 13 and Day 25. *Indicates significant changes ($p<0.05$) in DASS-21 scores on Day 13 and Day 25. PLA=Placebo; MB=Mushroom Blend; CAP=Capsules; BAR=Nutrition Bar. Significant changes in DASS-21 scores on Day 13 and Day 25 as compared to Baseline (Day 0) were identified when Mushroom Blend (MB) capsule (a) or snack bar (b) were consumed twice a day for a total of 250 mg supplementation. A decrease in scores suggests an improvement in psychological well-being, indicating reduced severity of depression, anxiety, or stress symptoms.

Table 4: PSQI Time course scores.

	PLA-CAP	MB-CAP	PLA-BAR	MB-BAR
Day 0	9.40 ± 1.82 n=20	13.00 ± 2.00 n=20	8.85 ± 1.73 n=20	10.85 ± 2.37 n=20
DAY 13	8.45 ± 1.76 n=20	10.55 ± 1.90* n=20	8.00 ± 1.73 n=19	9.24 ± 2.44* n=17
Day 25	7.80 ± 1.67* n=20	8.15 ± 1.35* n=20	7.22 ± 1.63* n=18	7.35 ± 1.93* n=17

Note: Presents PSQI scores (Mean ± SD) from baseline at Day 13 and 25. * Indicates significant changes ($p < 0.05$) in PSQI scores on Day 13 and 25 as compared to Baseline (Day 0). PLA=Placebo; MB=Mushroom Blend; CAP = Capsules; BAR = Nutrition Bar

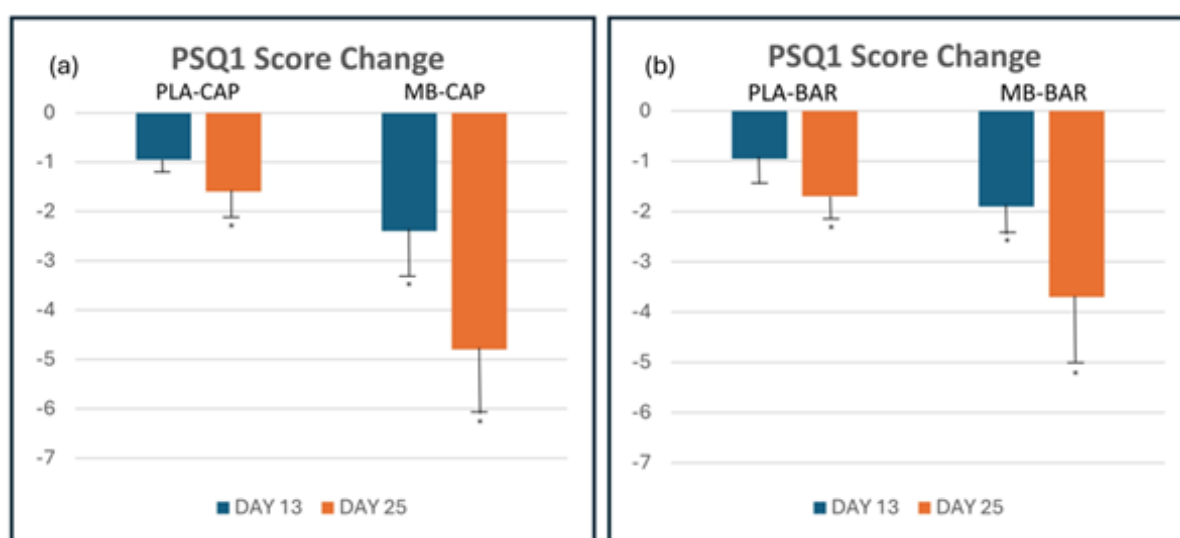


Figure 3: Presents changes in PSQI scores (Mean ± SD) from baseline at Day 13 and Day 25. *Indicates significant changes ($p < 0.05$) in PSQI scores on Day 13 and Day 25. PLA=Placebo; MB=Mushroom Blend; CAP=Capsules; BAR=Nutrition Bar. Significant changes ($p < 0.05$) in PSQI scores on Day 13 and Day 25 as compared to Baseline (Day 0) were identified when Mushroom blend (MB) capsule (a) or snack bar (b) were consumed twice a day for a total of 250 mg supplementation. Change in PSQI scores reflects shifts in overall sleep quality, where a decrease in score indicates overall improvement (better duration, efficiency, latency, and fewer disturbances).

Table 5: Salivary cortisol Time course scores.

	PLA-CAP	MB-CAP	PLA-BAR	MB-BAR
Day 0 Morning	5.70 ± 2.07 n=20	6.87 ± 1.73 n=20	4.91 ± 1.48 n=20	5.65 ± 1.96 n=20
Day 0 Evening	5.29 ± 2.04 n=20	6.43 ± 1.71 n=20	4.53 ± 1.48 n=20	5.19 ± 1.93 n=20
Day 25 Morning	5.63 ± 2.07* n=20	5.73 ± 1.47* n=20	4.87 ± 1.52* n=18	4.68 ± 1.40* n=17
Day 25	5.23 ± 2.05* n=20	5.21 ± 1.48* n=20	4.53 ± 1.51* n=18	4.26 ± 1.31* n=17

Note: *= Significant reduction ($p < 0.001$) in salivary cortisol measures (Mean ± SD) on Day 25 morning and evening vs Day 0 morning for all treatment groups. PLA=Placebo; MB=Mushroom Blend; CAP=Capsules; BAR=Nutrition Bar

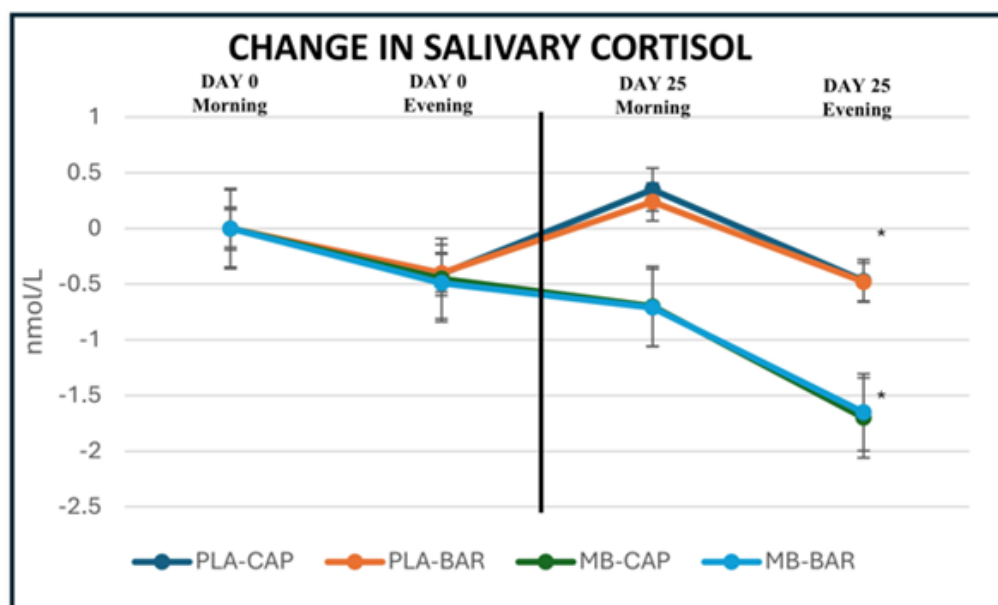


Figure 4: Salivary cortisol measurements (Mean ± SD) at Day 0 and Day 25 morning and evening. * Indicates significant reduction ($p < 0.001$) in salivary cortisol (PLA=Placebo; MB=Mushroom Blend; CAP=Capsules; BAR=Nutrition Bar). A significant reduction ($p < 0.001$) in salivary cortisol on Day 25 morning and evening vs Day 0 morning when Mushroom Blend (MB) capsule (green line) or snack bar (light blue line) compared to placebo capsule (dark blue line) or snack bar (orange line) were consumed twice a day for a total of 250 mg supplementation. The data suggests that MB supplementation led to a greater reduction in both morning and evening cortisol levels, compared to placebo. This points to a potential stress-relieving effect of mushroom extracts over the 25-day intervention.

Table 6: Oral temperature and cardiovascular assessment.

		PLA-CAP	MB-CAP	PLA-BAR	MB-BAR
Oral Temperature (°C)	Day 0	37.01 ± 0.04 n=20	36.99 ± 0.28 n=20	37.11 ± 0.31 n=20	37.08 ± 0.21 n=20
	Day 25	37.06 ± 0.23 n=20	37.02 ± 0.32 n=20	37.14 ± 0.44 n=19	36.94 ± 0.24 n=17
Heart Rate (beats per minute)	Day 0	76.40 ± 4.60 n=20	76.40 ± 4.50 n=20	77.75 ± 4.13 n=20	77.45 ± 4.74 n=20
	Day 25	76.80 ± 5.40 n=20	77.15 ± 4.20 n=20	77.26 ± 4.17 n=19	77.18 ± 7.77 n=17
Systolic Blood Pressure (mm Hg)	Day 0	116.9 ± 7.85 n=20	121.0 ± 7.12 n=20	115.6 ± 6.30 n=20	117.2 ± 8.03 n=20
	Day 25	118.3 ± 5.45 n=20	119.5 ± 7.59 n=20	117.0 ± 5.71 n=19	114.4 ± 6.59 n=17
Diastolic Blood Pressure (mm Hg)	Day 0	77.50 ± 7.52 n=20	77.05 ± 7.17 n=20	76.65 ± 6.70 n=20	77.35 ± 8.32 n=20
	Day 25	76.80 ± 6.96 n=20	74.75 ± 5.50 n=20	78.33 ± 6.86 n=19	77.35 ± 7.93 n=17

Note: Mean ± SD

PLA-CAP-Placebo Capsule | PLA-BAR-Placebo Bar

MB-CAP-Mushroom Blend Capsule | MB-BAR-Mushroom Blend Bar

Hematology, serum enzymes and creatinine

The results of baseline (Day 0) and final assessments (Day 25) for hematology measures of red and white blood integrity and status are presented in Tables 7 and 8. In addition, no significant

differences were determined in the change in hematology and serum liver enzymes and creatinine from baseline (Day 0) to final assessments (Day 25) and are considered supportive of short-term tolerance and general safety.

Table 7: Haematology assessments.

		PLA-CAP	MB-CAP	PLA-BAR	MB-BAR
Basophils (%)	Day 0	0.55 ± 0.51 n=20	0.45 ± 0.51 n=20	0.35 ± 0.49 n=20	0.30 ± 0.47 n=20
	Day 25	0.35 ± 0.49 n=20	0.35 ± 0.49 n=20	0.44 ± 0.51 n=18	0.35 ± 0.49 n=17
Eosinophils (%)	Day 0	3.70 ± 1.42 n=20	3.40±1.23 n=20	3.65 ± 1.46 n=20	3.85 ± 1.42 n=20
	Day 25	3.80 ± 1.32 n=20	3.35 ± 1.50 n=20	3.39 ± 1.58 n=18	3.71 ± 1.53 n=17
Hemoglobin (gms/dl)	Day 0	14.15 ± 1.40 n=20	14.62 ± 1.44 n=20	14.57 ± 1.34 n=20	14.14 ± 1.33 n=20
	Day 25	14.26 ± 1.23 n=20	14.67 ± 1.49 n=20	14.67 ± 1.01 n=18	14.09 ± 1.48 n=17
Lymphocytes (%)	Day 0	25.35 ± 3.15 n=20	27.25 ± 5.09 n=20	26.45 ± 5.17 n=20	28.35 ± 7.04 n=20
	Day 25	27.20 ± 5.19 n=20	28.25 ± 5.65 n=20	27.28 ± 5.10 n=18	26.06 ± 4.46 n=17
MCH (Pg)	Day 0	31.34 ± 5.61 n=20	32.01 ± 3.05 n=20	33.35 ± 3.24 n=20	32.72 ± 3.06 n=20
	Day 25	32.75 ± 4.51 n=20	32.55 ± 3.04 n=20	32.17 ± 4.53 n=18	32.90 ± 3.15 n=17
MCHC (Pg)	Day 0	34.14 ± 2.47 n=20	34.40 ± 2.24 n=20	33.67 ± 1.77 n=20	34.30 ± 1.68 n=20
	Day 25	33.9 2± 1.62 n=20	34.28±1.72 n=20	33.97±1.76 n=18	34.32 ± 2.40 n=17
MCV (fL)	Day 0	81.8 0± 6.49 n=20	83.99 ± 6.47 n=20	83.67 ± 4.54 n=20	84.13 ± 4.82 n=20
	Day 25	82.83 ± 5.70 n=20	83.45 ± 4.99 n=20	83.95 ± 4.87 n=18	84.42 ± 5.69 n=17
Monocytes (%)	Day 0	5.75 ± 1.68 n=20	5.75 ± 1.89 n=20	6.1 5± 1.63 n=20	5.90 ± 1.52 n=20
	Day 25	5.75±1.59 n=20	5.75 ± 1.80 n=20	6.28 ± 1.78 n=18	6.12 ± 1.54 n=17
Neutrophils (%)	Day 0	64.6 5± 3.60 n=20	63.15 ± 4.79 n=20	63.35 ± 4.84 n=20	61.95 ± 6.43 n=20
	Day 25	62.90 ± 4.40 n=20	62.30 ± 5.15 n=20	62.61 ± 5.47 n=18	63.76 ± 4.53 n=17
PCV (%)	Day 0	44.98 ± 5.29 n=20	45.76 ± 5.89 n=20	45.9 2± 5.36 n=20	44.97 ± 6.47 n=20
	Day 25	45.72 ± 5.14 n=20	44.96 ± 5.75 n=20	45.61 ± 5.41 n=18	44.71 ± 6.02 n=17
Platelet Count (103/ mm ³)	Day 0	271.5 ± 48.5 n=20	298.7 ± 65.6 n=20	291.7 ± 52.8 n=20	273.7 ± 55.4 n=20
	Day 25	285.6 ± 43.9 n=20	311.4 ± 68.54 n=20	291.5 ± 55.2 n=18	276.1 ± 43.8 n=17
R.B.C Count (109/ mm ³)	Day 0	5.27 ± 0.70 n=20	5.24 ± 0.82 n=20	5.22 ± 0.69 n=20	5.07 ± 0.76 n=20
	Day 25	5.07 ± 0.68 n=20	5.10 ± 0.78 n=20	5.48 ± 0.70 n=18	5.03 ± 0.52 n=17
RDW-CV (%)	Day 0	13.58 ± 1.62 n=20	13.94 ± 1.25 n=20	14.09 ± 1.42 n=20	13.87 ± 1.50 n=20
	Day 25	14.05 ± 1.50 n=20	13.99 ± 1.37 n=20	14.27 ± 1.31 n=18	13.89 ± 1.67 n=17
WBC Count 103/mm ³	Day 0	7.66 ± 1.12 n=20	7.94 ± 1.72 n=20	7.59 ± 1.59 n=20	7.59 ± 1.29 n=20
	Day 25	7.84 ± 1.09 n=20	7.85 ± 1.59 n=20	7.51 ± 1.37 n=18	7.79 ± 1.51 n=17

Note: Mean ± SD

PLA-CAP-Placebo Capsule | PLA-BAR-Placebo Bar

MB-CAP-Mushroom Blend Capsule | MB-BAR-Mushroom Blend Bar

Table 8: Liver function assessments.

		PLA-CAP	MB-CAP	PLA-BAR	MB-BAR
Alanine Transaminase (ALT/SGPT) (IU/I)	Day 0	33.80 ± 7.05 n=20	32.80 ± 6.14 n=20	33.95 ± 7.22 n=20	30.90±8.16 n=20
	Day 25	33.50±5.67 n=20	33.80±7.26 n=20	33.17±6.21 n=18	32.35±8.43 n=17
Aspartate Aminotransferase (AST/SGOT) (IU/I)	Day 0	35.60±7.49 n=20	39.80±3.79 n=20	35.35±8.96 n=20	34.95±8.18 n=20
	Day 25	36.10±6.50 n=20	39.60±4.86 n=20	35.28±6.96 n=18	37.00±6.02 n=17
Serum creatinine (mg/dl)	Day 0	0.94±0.26 n=20	0.98±0.30 n=20	0.91±0.26 n=20	0.88±0.25 n=20
	Day 25	0.94±0.25 n=20	0.95±0.21 n=20	0.93±0.22 n=18	0.99±0.25 n=17

Note: Mean ± SD

PLA-CAP-Placebo Capsule | PLA-BAR-Placebo Bar

MB-CAP-Mushroom Blend Capsule | MB-BAR-Mushroom Blend Bar

DISCUSSION

The present clinical trial evaluated the effects of a 25-day intervention with a proprietary organic Mushroom Blend (MB) delivered in either capsule or nutrition bar form on mood, sleep and stress-related biomarkers in healthy adults. In addition to assessing efficacy, the study included a broad evaluation of clinical safety markers to establish short-term tolerability. The experimental design of this investigation is unique in that it provided time-course comparison within the treatment groups compared to placebo and allowed for insight into different delivery forms, either capsules or snack bar. The results of the present investigation suggest that the specific organic mushroom blend utilized can have a general positive impact on sleep quality influencing mood states of sadness, stress and anxiety.

Mood improvements

Despite some baseline differences in DASS-21 scores due to the modestly sized trial, the organic mushroom blend was associated with statistically significant improvements in mood states, including reductions in sadness, anxiousness and stress. Both delivery formats (capsule and bar) produced meaningful improvements by Day 13, which were sustained or enhanced by Day 25. Importantly, while all groups demonstrated some degree of mood improvement over time the degree of change in both MB groups was significantly greater than in the placebo controls. This suggests a treatment-specific benefit that may reflect the bioactive compounds in the mushroom blend, such as adaptogenic polysaccharides, triterpenes, or other neuroactive constituents previously linked to modulation of the HPA axis [32].

Sleep quality enhancements

Sleep quality also improved significantly in the MB treatment arms compared to placebo as measured by the PSQI questionnaire. Notably, the MB-CAP and MB-BAR groups began the trial with higher baseline PSQI scores, indicating poorer initial sleep quality. However, both groups experienced marked reductions in PSQI scores by Day 13, with further improvements by Day 25, suggesting rapid and sustained benefits. These results are consistent with

emerging evidence that certain medicinal mushrooms may influence sleep experiences and subjective sleep quality *via* modulation of neurotransmitters such as GABA and serotonin, or through indirect effects on stress reactivity and inflammation [9, 33-35].

Salivary cortisol reductions

Reductions in salivary cortisol levels observed in this study further support the stress-relieving potential of the mushroom blend. While all treatment groups demonstrated significant decreases in both morning and evening cortisol by Day 25, the magnitude of change was notably greater in the MB treatment groups. These physiological changes parallel the improvements observed in DASS-21 scores, suggesting a coherent relationship between reduced cortisol output and improvements in mood and perceived stress. Although some baseline differences in cortisol levels were observed, variability is not uncommon in stress biomarker studies and likely reflects individual differences in HPA axis activity rather than a flaw in study design. Importantly, previous research validates the use of cortisol as a meaningful biomarker by establishing its predictable diurnal rhythm, with levels typically peaking in the early morning and declining throughout the day [36]. This rhythm plays a regulatory role in many physiological systems, including the sleep-wake cycle, stress reactivity and immune function, thereby reinforcing the biological relevance of monitoring salivary cortisol in the current study. While the relationship between cortisol and sleep remains complex, several studies have shown that poor sleep quality and insomnia can be associated with altered cortisol patterns, either elevated evening levels or reduced morning responses [37,38]. These findings help interpret our own data, which showed improved PSQI scores alongside normalized cortisol levels, particularly in MB treatment groups. Additionally, evidence suggesting that higher morning cortisol levels may be associated with shorter sleep durations (less than five hours) supports the value of targeting cortisol modulation in interventions aimed at improving sleep and stress outcomes [39]. Collectively, these prior findings enhance the interpretive strength of our results, suggesting that the MB's beneficial effects on psychological well-being may be mediated, at least in part, through its regulatory influence on the HPA axis [40].

Safety and tolerability

MB supplementation for 25 days did not result in changes in oral temperature, heart rate, blood pressure, hematological measures or serum liver-based enzymes or creatinine. Adverse events were monitored systematically throughout the study using open-ended questioning and safety assessments, including vital signs and hematological measures. Two participants (one from GS-BAR and one from PLA-BAR) withdrew before study completion due to personal reasons and were excluded from the final Day 25 analysis. No serious adverse events attributable to the study product were observed. These observational measures, combined with no major reported adverse effects, are suggestive that short-term supplementation of the applied mushroom blend is well tolerated and generally safe.

Study strengths

Strengths of the study include the randomized, double-blind, placebo-controlled design, as this minimizes bias and increases internal validity. The inclusion of capsule and functional snack bar delivery formats provides practical insight into consumer-relevant supplementation strategies. Objective biomarkers (salivary cortisol) alongside validated psychological and sleep assessment tools (DASS-21 and PSQI) enhance the robustness of outcome measures. Importantly, the incorporation of stress-related biomarkers anchors the findings beyond self-report measures, partially counter-balancing the absence of strict dietary or exercise controls. Rather than a limitation, this design reflects real-world consumer conditions under which such products are typically used, thereby strengthening ecological validity. Furthermore, the safety assessments (vital signs and hematological analyses) support the short-term tolerability of the intervention.

Study limitations and future directions

Limitations of the present study include the modest sample size ($n=20$ per group) and the parallel, rather than cross-over, design, which limits statistical power. Although participants were randomized, some baseline differences were observed across outcome measures. This variability is common in smaller randomized trials and likely reflects natural sample variation rather than systematic imbalance. Within-group analyses were appropriately applied, but future work could benefit from baseline-adjusted models (e.g., ANCOVA) or reporting percent change from baseline to improve between-group comparisons.

Improvements seen in the placebo groups suggest a potential placebo effect, likely influenced by factors such as response bias, co-intervention bias, desire for change, emotional support, or observation effects, as well as diet or lifestyle shifts outside of the trial [41]. While dietary and exercise patterns were not controlled, this design more closely reflects the way consumers actually use such products in daily life. Importantly, the inclusion of a stress biomarker (salivary cortisol) helps to anchor findings beyond self-report and partially offsets these uncontrolled variables.

Other considerations include the absence of objective sleep and mood monitoring, the short intervention period and the limited demographic and geographic diversity of the sample. Even with these limitations, the findings provide encouraging preliminary evidence that short-term use of the mushroom blend may support sleep quality and mood in young adults.

CONCLUSION

Supplementation in either capsule or snack bar form of a commercially available, organic, mushroom blend consisting of organic *Ganoderma lucidum*, organic *Cordyceps militaris* fruiting body extract, organic *Hericium erinaceus* fruiting body extract and organic fermented *Cordyceps sinensis* mycelium extract may positively influence mood state, sleep quality and salivary cortisol levels. These mushrooms are rich in adaptogenic compounds such as β -glucans, triterpenes and cordycepin, which support stress resilience, emotional well-being and immune function. The observed benefits are consistent with existing research on Lion's Mane for cognitive and mood support, Cordyceps for energy and metabolic regulation and Reishi for calming and neuroendocrine balance. Moreover, the onset of potential benefits can occur within two weeks and continue to show improvements. The availability of both capsule and nutritional snack bar formats also provides greater flexibility for daily use. Lastly, general physical assessments combined with blood indices support the safety and tolerability of supplementation with this adaptogenic mushroom blend.

SUPPLEMENTARY DATA AND AVAILABILITY

No supplementary materials are associated with this article. De-identified raw datasets generated and analyzed during the study are available from the corresponding author upon reasonable request and with approval from the Institutional Ethics Committee.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest. RNV, RW and MH report consulting and/or advisory roles with NURA USA LLC. MKR is an employee of NURA USA LLC. All other authors declare no competing interests. The funders had a role in the design of the study; in the writing of the manuscript; and in the decision to publish the results. The funders had no role in the collection, analyses and interpretation of data.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and the protocol received approval on January 29, 2024, from the Sri B.M. Patil Medical College Hospital's Institutional Ethics Committee (IEC) of Shri B. M. Patil Medical College, Hospital & Research Centre, Vijayapura, Karnataka, India. The IEC functions as an independent body

representing the institution, research participants and the wider community and is recognized by the Department of Health Research, Government of India. All research involving human participants at this institution is governed by the Indian Council of Medical Research (ICMR) National Ethical Guidelines, which align with international standards. The trial was registered with the Clinical Trials Registry-India (CTRI/2024/02/063100).

INFORMED CONSENT STATEMENT

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

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