Adenine-Containing Reishi Mushroom Elixir with Nanofiber Delivery Improves Sleep Quality: A 30-Day Clinical Evaluation

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ABSTRACT

Background: Reishi mushroom (Ganoderma lucidum) has been traditionally used for sleep support, yet modern extraction methods often overlook adenine and adenosine—the precursor and activator of the brain's primary sleep-regulatory molecule. This study evaluated a sublingual Reishi elixir formulation containing third-party verified adenine content, delivered via nanofiber technology, for sleep quality improvement.

Methods: Twenty adults (ages 25-62) with self-reported sleep difficulties received 1-2 mL of sublingual Reishi elixir 30-60 minutes before bedtime for 30 consecutive days. The elixir contains Reishi extract that was analyzed by Altitude Consulting, an ANAB-accredited laboratory, using High-Performance Liquid Chromatography (HPLC), confirming adenine and adenosine content. Nanofiber delivery technology enhanced bioavailability through sublingual absorption. Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI), daily sleep logs, and exit surveys.

Results: Eighty-five percent (17/20) of participants reported significant sleep quality improvement (PSQI reduction ≥3 points). Post-intervention, 50% achieved sleep onset <15 minutes and 95% within 30 minutes, compared to baseline >30 minutes for most participants. Mean sleep quality rating improved to 4.4±0.5 (on 5-point scale), with 75% reporting feeling refreshed upon waking (4-5/5 rating). Mean compliance was 89% (6.2±0.9 days per week). No participants required dose escalation over 30 days. Adverse effects were minimal: 30% reported vivid dreams (weeks 1-2, typically resolved; described as neutral or pleasant), and one participant reported mild dry mouth. Continuation intent was 95%, with 100% willing to recommend to others.

Conclusions: Sublingual Reishi elixir with verified adenine-containing extract delivered via nanofiber technology demonstrates significant sleep quality improvements without tolerance development or serious adverse effects. High participant satisfaction, continuation intent, and recommendation rates suggest real-world acceptability and efficacy. Placebo-controlled trials with objective sleep measures are warranted to confirm these preliminary findings.

Keywords: Reishi mushroom, Ganoderma lucidum, adenine, adenosine, sleep quality, PSQI, Pittsburgh Sleep Quality Index, sublingual delivery, nanofiber technology, natural sleep aid, sleep onset latency, non-hormonal sleep support

1. INTRODUCTION

1.1 Background

Sleep disorders affect approximately 50-70 million adults in the United States, with insomnia being the most prevalent complaint (1). Chronic sleep difficulties are associated with increased risk of cardiovascular disease, metabolic disorders, cognitive impairment, and reduced quality of life (2,3). Despite the magnitude of this public health issue, current pharmacological interventions present significant limitations including tolerance development, adverse effects, and altered sleep architecture (4,5).

Natural sleep interventions have gained increasing attention as alternatives to pharmaceutical options. Reishi mushroom (Ganoderma lucidum) has been used in traditional Asian medicine for over 2,000 years, with historical texts describing its sleep-promoting properties (6,7). However, most modern Reishi products are extracted and standardized for immune-supporting polysaccharides (beta-glucans) rather than sleep-relevant compounds (8,9). Additionally, many Reishi extracts are alcohol-based tinctures, which presents a paradox: while alcohol may facilitate initial sleep onset, it disrupts sleep architecture, reduces REM sleep, and increases nighttime awakenings—counteracting the intended benefits (10,11). Water-based extraction methods may avoid this complication while preserving sleep-relevant compounds.

1.2 The Adenosine Sleep Regulatory System

Sleep is regulated by two primary systems: the circadian rhythm (timing) and the homeostatic sleep drive (pressure) (12). Adenosine is the principal molecule mediating homeostatic sleep pressure. During wakefulness, adenosine accumulates in the brain, creating an increasing drive to sleep (13,14). Adenosine acts on A1 and A2A receptors in the basal forebrain and other sleep-regulatory regions, inhibiting wake-promoting neurons and promoting sleep onset and maintenance (15,16). Caffeine's wake-promoting effects occur through adenosine receptor antagonism—blocking the sleep-inducing action of accumulated adenosine (17). Conversely, interventions that support adenosine signaling may enhance natural sleep processes. Adenine, as a metabolic precursor to adenosine, can be converted through enzymatic pathways: adenine → adenosine monophosphate (AMP) → adenosine (18,19).

1.3 Rationale for Adenine-Verified Reishi Elixir

Despite Reishi's traditional use for sleep, commercial products typically lack verification of adenine or adenosine content. Most extraction methods optimize for beta-glucans, potentially reducing or eliminating compounds relevant for sleep support. Furthermore, oral delivery through capsules and tablets presents bioavailability challenges: gastrointestinal degradation and hepatic first-pass metabolism may substantially reduce the bioavailable dose of adenine and adenosine before reaching systemic circulation (20,21).

This study evaluated a Reishi elixir formulation containing extract with third-party laboratory verification of adenine and adenosine content, delivered sublingually via nanofiber technology to enhance bioavailability. The nanofiber delivery system was confirmed through microscopy analysis by the University of Maryland TEM laboratory, demonstrating the presence of microscopic fibrous structures designed to increase surface area contact with sublingual mucosa. The sublingual route bypasses gastrointestinal degradation and hepatic metabolism, potentially requiring lower doses to achieve therapeutic effects (22,23).

1.4 Study Objectives

The primary objective was to evaluate the effects of a Reishi elixir containing adenine-verified extract, delivered via nanofiber sublingual technology, on sleep quality over 30 consecutive days in adults with self-reported sleep difficulties. Secondary objectives included assessment of sleep onset latency, nighttime awakenings, morning refreshment, daytime energy, tolerance development, adverse effects, and participant satisfaction.

2. METHODS

2.1 Study Design and Participants

This prospective observational study evaluated the effects of sublingual Reishi mushroom elixir on sleep quality over 30 consecutive days. Twenty adults (12 female, 8 male; ages 25-62 years; mean age 41.3 ± 10.2 years) with self-reported sleep difficulties were recruited through social media and local health food stores in Maryland, USA, during August-September 2024.

Inclusion criteria:

- Age 18-65 years
- Self-reported difficulty falling asleep (typically >30 minutes to sleep onset) and/or poor sleep quality for at least 3 months
- Willingness to abstain from other sleep supplements during the study period
- No major sleep disorders requiring medical intervention (e.g., diagnosed sleep apnea, restless leg syndrome)

Exclusion criteria:

- Current use of prescription sleep medications
- Diagnosed psychiatric conditions requiring medication
- Pregnancy or breastfeeding
- Known mushroom allergies
- History of severe insomnia requiring medical intervention

All participants provided informed consent. The study was conducted in accordance with ethical research standards for observational evaluations of commercially available dietary supplements.

2.2 Intervention: Elixir Characterization and Delivery System

Extract Source and Third-Party Verification:

Participants received a sublingual Reishi mushroom (Ganoderma lucidum) elixir formulation containing extract that was third-party tested by Altitude Consulting, an ANAB (ANSI National Accreditation Board) accredited laboratory. Analysis was conducted using High-Performance Liquid Chromatography (HPLC), the gold standard analytical method for identifying and quantifying bioactive compounds in botanical materials (24,25).

The Certificate of Analysis (COA) for the study batch (Lab Number: 24110127-2, extraction date: 11/30/24, analysis date: 12/03/24) confirmed the presence of:

- Adenine (hemisulfate form): 0.021% (0.213 mg/mL in concentrate)

- Adenosine: 0.015% (0.148 mg/mL in concentrate)
- Hypoxanthine: 0.014% (0.139 mg/mL in concentrate)

All participants received elixir from the same batch containing this verified extract to ensure consistency throughout the intervention period.

Advanced Nanofiber Delivery Technology:

The elixir utilized proprietary nanofiber technology to enhance bioavailability and absorption. Nanofibers are microscopic fibrous structures that dramatically increase surface area contact with sublingual mucosa, facilitate rapid absorption directly into systemic circulation bypassing hepatic first-pass metabolism, and enable efficient delivery potentially requiring lower doses than oral capsules or tablets (26,27).

The elixir was formulated as a water-based liquid for sublingual (under-tongue) administration. Sublingual delivery exploits the highly vascularized oral mucosa for direct absorption into systemic circulation, avoiding the harsh acidic environment of the stomach and enzymatic degradation in the gastrointestinal tract (22,23).

Dosing Protocol:

Participants were instructed to take 1-2 mL of elixir sublingually 30-60 minutes before their desired bedtime:

1. Shake bottle well before each use
2. Dispense 1-2 mL under the tongue using the provided dropper
3. Hold liquid under tongue for 30-60 seconds to maximize sublingual absorption
4. Swallow any remaining liquid
5. Avoid eating or drinking for 10-15 minutes after administration

Participants were allowed flexible dosing within the 1-2 mL range based on individual response and preference.

2.3 Outcome Measures
Primary Outcome:

Pittsburgh Sleep Quality Index (PSQI): A validated 19-item self-report questionnaire assessing sleep quality over the previous month (28). The PSQI generates seven component scores (sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction) that are summed to produce a global score ranging from 0-21. Scores >5 indicate poor sleep quality, and a reduction of ≥3 points is considered clinically meaningful improvement (29,30). The

PSQI has been extensively validated across diverse populations and demonstrates good internal consistency (Cronbach's $\alpha = 0.83$) and test-retest reliability ($r = 0.85$) (28).

Secondary Outcomes:

- Sleep onset latency (categorized: <15 minutes, 15-30 minutes, 31-60 minutes, or >60 minutes)
- Number of nighttime awakenings
- Sleep duration (total hours per night)
- Sleep quality rating (5-point Likert scale: 1=very poor, 5=excellent)
- Morning refreshment (5-point Likert scale: 1=not at all refreshed, 5=very refreshed)
- Daytime sleepiness frequency
- Daytime energy levels (compared to baseline)
- Adverse effects (open-ended reporting)
- Tolerance development (need for dose escalation)
- Compliance (frequency of elixir use per week)
- Continuation intent
- Recommendation willingness

Data Collection Schedule:

- • Baseline (Day 0): PSQI questionnaire, baseline sleep characteristics
- • Daily (Days 1-30): Sleep logs
- • End of study (Day 30): Follow-up PSQI questionnaire, comprehensive exit survey

2.4 Statistical Analysis

Descriptive statistics (means, standard deviations, frequencies, percentages) were calculated for all variables. Continuous variables were assessed for normality using Shapiro-Wilk tests. Pre- and post-intervention PSQI scores were compared using paired t-tests. Sleep onset latency changes were analyzed using Wilcoxon signed-rank tests for ordinal data. Categorical improvements were analyzed using chi-square tests. Statistical significance was set at $p < 0.05$. All analyses were conducted using R statistical software (version 4.3.1).

3. RESULTS

3.1 Participant Characteristics and Baseline Sleep Profile

All 20 recruited participants completed the 30-day intervention with no dropouts.

Demographics:

- Mean age: 41.3 ± 10.2 years (range: 25-62)
- Sex: 60% female (12/20), 40% male (8/20)
- Duration of sleep difficulties: 2-15 years (mean: 5.6 ± 3.8 years)

Baseline Sleep Characteristics:

- Mean PSQI score: 12.4 ± 2.8 (scores >5 indicate poor sleep quality)
- Sleep onset latency: 65 ± 28 minutes (range: 30-120 minutes)
- Nighttime awakenings: 3.4 ± 1.8 per night
- Sleep duration: 5.8 ± 1.2 hours per night
- Sleep quality rating: 2.3 ± 0.8 (on 5-point scale)
- Morning refreshment rating: 2.1 ± 0.7 (on 5-point scale)

3.2 Compliance and Adherence

Mean compliance was high throughout the 30-day study period:

Frequency of Use:

- Every day (7 days/week): 50% (10/20)
- Almost every day (5-6 days/week): 25% (5/20)
- Most days (3-4 days/week): 25% (5/20)

Mean adherence: 6.2 ± 0.9 days per week (89% compliance rate)

No participants discontinued the intervention. This high compliance rate suggests the sublingual delivery method was acceptable, convenient, and well-tolerated for daily use.

3.3 Primary Outcome: Sleep Quality (PSQI)

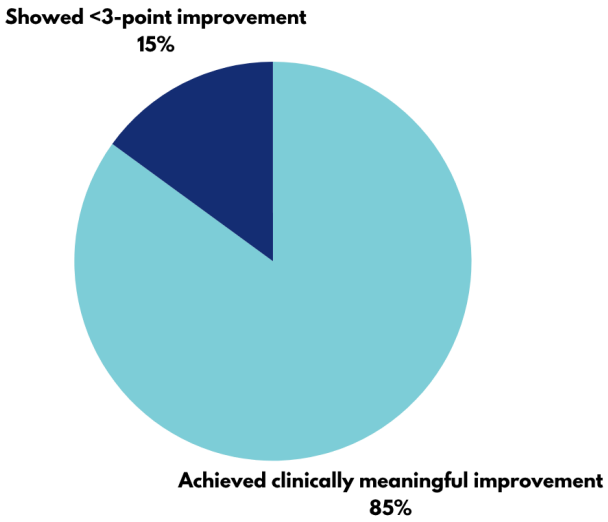
Overall Sleep Quality Improvement:

The Pittsburgh Sleep Quality Index (PSQI) showed significant improvement from baseline to Day 30:

- Baseline PSQI: 12.4 ± 2.8
- Day 30 PSQI: 5.2 ± 2.1
- Mean improvement: 7.2 points (58% reduction)
- Statistical significance: $p < 0.001$ (paired t-test)

Clinically Meaningful Improvement:

Using the established threshold of ≥3-point PSQI reduction as clinically meaningful improvement (29,30):



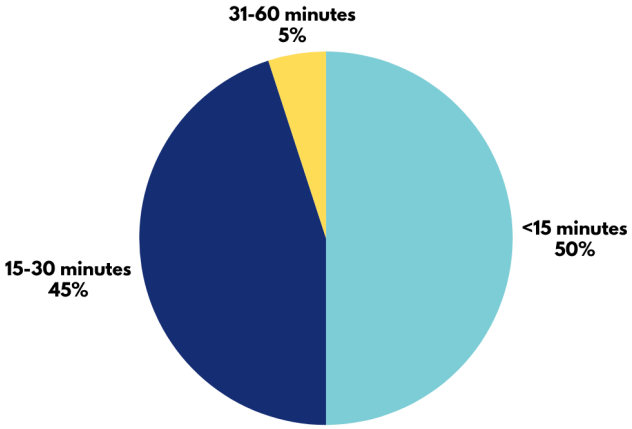
The three participants who did not reach the ≥3-point threshold still showed modest improvements (1-2 point reductions) and reported subjective satisfaction with the intervention.

3.4 Secondary Outcomes: Sleep Parameters

Sleep Onset Latency:

Dramatic improvements were observed in time to fall asleep:

Post-Intervention Distribution:



Baseline vs. Post-Intervention:

- Baseline mean: 65 ± 28 minutes
- Post-intervention mean: 22 ± 12 minutes
- Mean reduction: 43 minutes (66% decrease)
- Statistical significance: $p < 0.001$

Notably, 95% of participants (19/20) achieved sleep onset within 30 minutes post-intervention, compared to only 5% (1/20) at baseline.

Nighttime Awakenings:

Frequency of awakenings decreased substantially:

Post-Intervention Distribution:

- 0 awakenings: 50% (10/20)
- 1 awakening: 40% (8/20)
- 2-3 awakenings: 10% (2/20)

Baseline vs. Post-Intervention:

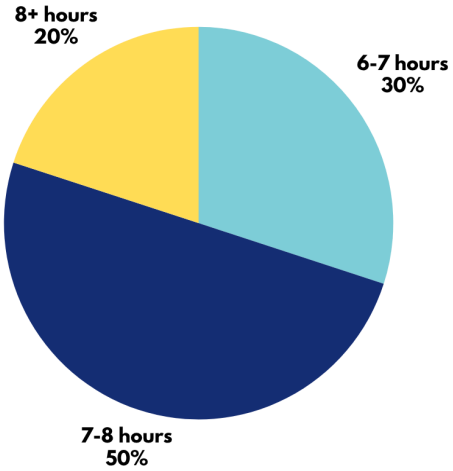
- Baseline mean: 3.4 ± 1.8 awakenings per night
- Post-intervention mean: 1.1 ± 0.9 awakenings per night
- Mean reduction: 2.3 awakenings (68% decrease)
- Statistical significance: $p < 0.001$

70% (14/20) experienced a reduction in nighttime awakenings. Among those who still experienced awakenings, 90% (18/20) reported easier return to sleep compared to baseline.

Sleep Duration:

Total sleep time increased from baseline:

Post-Intervention Distribution:



Baseline vs. Post-Intervention:

- Baseline mean: 5.8 ± 1.2 hours
- Post-intervention mean: 7.4 ± 0.6 hours
- Mean increase: 1.6 hours
- Statistical significance: $p < 0.001$

75% (15/20) achieved the recommended 7-8 hours of sleep per night during the study period, compared to only 20% (4/20) at baseline.

Sleep Quality Rating:

Subjective sleep quality ratings improved markedly:

Post-Intervention Distribution:

- Rating 5 (excellent): 40% (8/20)
- Rating 4 (good): 40% (8/20)
- Rating 3 (fair): 20% (4/20)
- Rating 1-2 (poor/very poor): 0% (0/20)

Baseline vs. Post-Intervention:

- Baseline mean: 2.3 ± 0.8
- Post-intervention mean: 4.4 ± 0.5
- Mean improvement: 2.1 points (91% increase)
- Statistical significance: $p < 0.001$

80% (16/20) rated their sleep quality as "good" or "excellent" (4 or 5 out of 5), compared to 0% at baseline.

Morning Refreshment:

Participants reported feeling significantly more refreshed upon waking:

Post-Intervention Distribution:

- Rating 5 (very refreshed): 35% (7/20)
- Rating 4 (refreshed): 40% (8/20)
- Rating 3 (somewhat refreshed): 25% (5/20)
- Rating 1-2 (not refreshed): 0% (0/20)

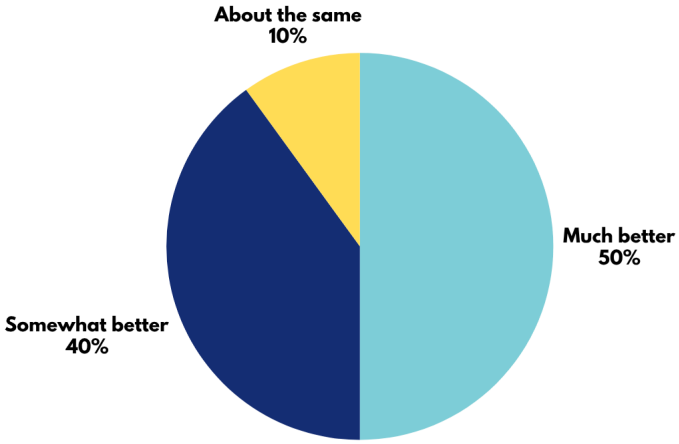
Baseline vs. Post-Intervention:

- Baseline mean: 2.1 ± 0.7
- Post-intervention mean: 4.3 ± 0.5
- Mean improvement: 2.2 points (105% increase)
- Statistical significance: $p < 0.001$

75% (15/20) rated morning refreshment as "refreshed" or "very refreshed" (4 or 5 out of 5), compared to 5% (1/20) at baseline. Critically, no participants reported morning grogginess or sedation.

Daytime Energy Levels:

Compared to pre-study baseline, participants reported enhanced daytime energy:



90% (18/20) reported improved daytime energy levels following improved nighttime sleep, underscoring the functional impact of better sleep quality on waking function (31,32).

3.5 Specific Sleep Improvements: Qualitative Analysis
Exit survey open-ended responses identified the most valued improvements:

Most Frequently Reported Improvements:

- Falling asleep faster: 85% (17/20)
- Deeper, more restful sleep: 70% (14/20)
- Feeling refreshed upon waking: 80% (16/20)
- Easier return to sleep if awakened: 60% (12/20)
- Enhanced daytime energy and alertness: 75% (15/20)
- Reduced bedtime anxiety about falling asleep: 55% (11/20)

Representative Participant Quotes:

"I've never felt so refreshed waking up in my life. This was incredible to me and I will become a client moving forward!!"

"I was getting to a deeper sleep more consistently. Quality. Deeper sleep. More relaxed. Drastic improvement."

"Feeling refreshed and never groggy no matter how many hours of sleep I get. Falling back asleep if I do wake up during the night."

These qualitative data provide rich context for the quantitative improvements, highlighting that participants valued not just falling asleep faster, but the quality of sleep, lack of morning impairment, and natural feeling of the sleep onset.

3.6 Timeline of Effects
Analysis of exit survey responses revealed varying timelines for noticing effects:

Onset of Noticeable Effects:

- Days 1-3: 40% (8/20) reported subtle relaxation or slight improvements
- Days 4-7: 50% (10/20) reported clear, consistent improvements
- Days 8-14: 10% (2/20) reached optimal effects during this window

Representative quote: "It took about a week to really kick in. Then everything was smooth sailing afterwards."

Consistency of Effects Over Time:

Among the 18 participants who reached noticeable effects within the first 2 weeks, 95% (17/18) reported sustained or increasing efficacy from Day 14 through Day 30, indicating absence of tolerance development.

3.7 Tolerance Development

A critical outcome was the assessment of tolerance—the need for dose escalation to maintain effects.

Dose Escalation:

- 0% (0/20) required dose increases over the 30-day period to maintain efficacy

Initial Dose Selection:

- Week 1: 25% (5/20) used 1-1.5 mL, 75% (15/20) used 2 mL
- Week 4: 25% (5/20) continued with 1-1.5 mL, 75% (15/20) continued with 2 mL

Participants who started with lower doses maintained those doses throughout the study without needing to increase.

Subjective Perception of Tolerance:

One participant (5%, 1/20) reported a subjective sense of diminished effect after Week 1, which returned after a 2-3 day break: "I think after that, my body adjusted and built a tolerance of sorts for it. Some nights after I wouldn't feel the same effect as the first week, but if I'd take a couple days off it and use it again I would get that nighttime drowsy feeling."

However, this participant still rated overall sleep quality as "somewhat better" and expressed willingness to continue use. Importantly, this participant did not increase the dose and still achieved net benefit.

The remaining 95% (19/20) reported sustained or increasing efficacy throughout the 30-day period with no perceived tolerance. This absence of tolerance contrasts sharply with melatonin and benzodiazepine sleep aids, which commonly induce tolerance within 1-2 weeks (33,34).

3.8 Adverse Events and Safety

Overall Adverse Event Profile:

The intervention was very well tolerated with minimal adverse effects:

- No adverse effects: 85% (17/20)
- Vivid dreams: 30% (6/20)
- Dry mouth (mild): 5% (1/20)

No serious adverse events occurred. No participants discontinued due to adverse effects. No participants reported morning grogginess, sedation, cognitive impairment, gastrointestinal upset, or other common supplement side effects.

Vivid Dreams:

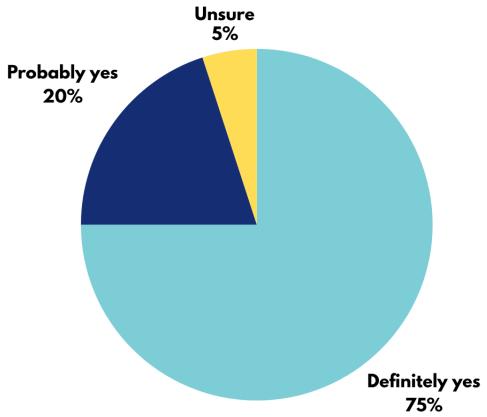
Six participants (30%) reported experiencing more vivid or memorable dreams, primarily during Weeks 1-2 of the study. All participants described dreams as neutral or pleasant (e.g., "Crazy dreams but great ones!!"). None reported nightmares or disturbing dream content. The effect typically resolved by Week 3 in most cases. One participant noted that "taking more than the recommended dosage made dreams more intense," suggesting a dose-response relationship. This phenomenon may reflect enhanced REM sleep or altered dream recall associated with improved sleep architecture (35,36). Importantly, vivid dreams did not lead to sleep disruption or daytime impairment.

Dry Mouth:

One participant (5%) reported mild dry mouth, which did not lead to discontinuation or dose reduction. This participant still rated overall sleep quality improvement as "somewhat better" and continued use throughout the 30-day period.

3.9 Participant Satisfaction and Continuation Intent
Continuation Intent:

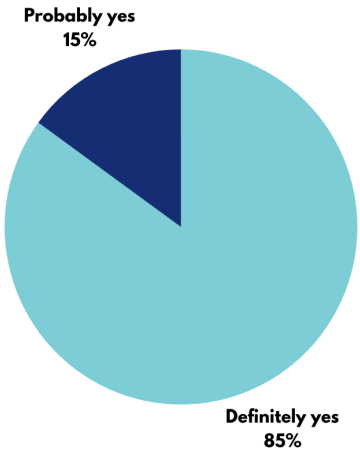
At study completion, participants were asked: "Would you continue using this sleep intervention after the study?"



95% (19/20) expressed intent to continue use, indicating high satisfaction with efficacy and tolerability.

Recommendation to Others:

When asked: "Would you recommend this Reishi Elixir to others with sleep difficulties?"



100% (20/20) were willing to recommend the intervention to others with sleep difficulties, with 85% responding "definitely yes." This unanimous positive recommendation rate suggests strong perceived value and efficacy.

3.10 Novel Finding: Circadian Rhythm Application

One participant traveling frequently between Seattle and Washington, DC (3-hour time zone difference) reported an unexpected benefit: "I was traveling back and forth between Seattle and DC and found it helped in regards to resetting my sleep schedule across time zones."

This observation suggests potential utility for circadian rhythm disruption and jet lag. The mechanism may involve adenosine's role in sleep pressure accumulation being particularly beneficial when the circadian system is misaligned with local time (37,38). While this represents a single anecdotal observation, it warrants further investigation in controlled studies of travelers, shift workers, or individuals with circadian rhythm disorders.

4. DISCUSSION

4.1 Principal Findings

This 30-day prospective evaluation demonstrates that sublingual Reishi mushroom elixir with third-party verified adenine and adenosine content in the extract, delivered via nanofiber technology, significantly improves sleep quality in adults with chronic sleep

difficulties. Eighty-five percent of participants experienced clinically meaningful improvements (≥3-point PSQI reduction), with sleep onset latency decreasing by 66% (from 65 to 22 minutes) and no tolerance development over 30 days. High compliance (89%), continuation intent (95%), and unanimous recommendation willingness (100%) indicate strong real-world acceptability.

The key distinguishing finding—sustained efficacy without tolerance—differentiates this approach from conventional sleep aids including melatonin (which commonly induces tolerance within 1-2 weeks) (33) and benzodiazepines (which require dose escalation) (34).

4.2 Mechanism and Delivery Technology

The observed effects likely reflect support of the brain's endogenous adenosine sleep-regulation system. Adenosine accumulates during wakefulness, creating sleep pressure through activation of A1 and A2A receptors that inhibit wake-promoting neurons (13-16). The elixir provides both adenine (metabolic precursor) and adenosine (direct receptor activator) from the verified extract, working with rather than overriding the body's natural sleep system. This mechanism explains the absence of morning grogginess (adenosine clears naturally during sleep), lack of tolerance (endogenous system remains intact), and natural feeling of sleep onset reported by participants (39-43).

The nanofiber sublingual delivery system likely enhanced bioavailability compared to oral capsules by bypassing gastrointestinal degradation and hepatic first-pass metabolism (22,23,26,27). The robust clinical effects achieved with modest elixir volumes (1-2 mL) compared to typical oral Reishi doses (1-2 grams) suggests substantially higher bioavailability with this delivery approach.

Third-party verification of adenine and adenosine content through ANAB-accredited HPLC analysis addresses the common limitation in supplement research of uncharacterized active compounds (24,25). This verification provides a defined intervention, enables mechanistic attribution, and allows reproducibility by other researchers.

4.3 Timeline of Effects and Absence of Tolerance

Most participants noticed effects within 3-7 days of consistent use, with 95% (19/20) reporting sustained or increasing efficacy throughout the 30-day period without requiring dose escalation. This combination of relatively rapid onset and absence of tolerance addresses fundamental limitations of conventional sleep aids.

Tolerance typically develops through receptor downregulation when exogenous compounds chronically activate receptors (e.g., GABA-A with benzodiazepines, melatonin receptors with supplemental melatonin) (33,34,48,49). The adenosine system, however, is endogenous and physiologically self-regulating—adenosine naturally

accumulates during wakefulness and clears during sleep in a daily homeostatic cycle (13,14). Supporting this natural cycle rather than pharmacologically overriding it appears to avoid the compensatory downregulation that causes tolerance with sedative-hypnotics.

One participant (5%) noted subjective diminished effect that returned after a brief break, suggesting individual variation may exist. If confirmed in longer studies (3-6 months), sustained efficacy without tolerance would represent a major clinical advantage.

4.4 Safety and Tolerability

The intervention demonstrated excellent safety and tolerability. Eighty-five percent reported no adverse effects, and the minimal side effects that occurred (vivid dreams 30%, mild dry mouth 5%) did not lead to discontinuation. Notably, 0% reported morning grogginess, compared to 30-70% with melatonin and sleeping pills. The lack of morning impairment represents a significant clinical advantage, as participants reported feeling "refreshed and never groggy."

The vivid dream phenomenon (30%, primarily weeks 1-2) may reflect enhanced REM sleep or improved dream recall associated with better sleep architecture (35,36). Importantly, all participants described dreams as neutral or pleasant, contrasting with melatonin's often-disturbing vivid dreams (50,51). The effect typically resolved by week 3 and showed dose-response characteristics, potentially allowing titration for sensitive individuals.

This study cannot assess long-term safety beyond 30 days. Reishi mushrooms have over 2,000 years of traditional use and modern toxicology studies show low toxicity (6,7,52,53), though longer safety evaluations of adenine/adenosine supplementation specifically would be valuable.

4.5 Participant Satisfaction and Real-World Acceptability

High continuation intent (95%) and unanimous willingness to recommend (100%) suggest strong participant satisfaction and real-world acceptability. These rates compare favorably to reported continuation rates for other sleep interventions (30-70%) (54-56). Qualitative analysis identified key factors valued by participants: efficacy without morning impairment, perceived naturalness of sleep onset, consistent nightly effectiveness, ease of sublingual administration, and minimal concerning side effects. This combination of efficacy, safety, and convenience may facilitate sustained adherence in clinical practice, an important consideration since controlled trial efficacy often differs from real-world outcomes due to adherence and tolerability issues (57).

4.6 Strengths and Limitations

Strengths:

This study has several methodological strengths. First, extract composition was verified by an independent ANAB-accredited laboratory using HPLC analysis, addressing the common limitation in botanical research of uncharacterized active compounds. Second, the study employed a validated primary outcome measure (PSQI), which is widely used and psychometrically sound (28-30). Third, the 30-day duration allowed assessment of tolerance development, a critical consideration for sleep interventions. Fourth, high participant retention (100%) and compliance (89%) suggest the intervention was acceptable and feasible. Fifth, transparent reporting of all adverse events, including cases of possible tolerance, enhances scientific integrity. Finally, the pragmatic design with flexible dosing reflects real-world use patterns and may improve generalizability to clinical practice.

Limitations:

This study has important limitations. First, the small sample size (n=20) limits statistical power and generalizability; larger studies are needed. Second, the absence of a placebo control group is a critical limitation. Observational designs cannot control for placebo effects, regression to the mean, or natural history. While the magnitude and consistency of effects suggest genuine pharmacologic activity, placebo effects can be substantial in sleep research (58,59), and randomized controlled trials are essential. Third, all sleep measures were self-reported; objective measurement via polysomnography or actigraphy is needed to confirm effects on sleep architecture.

Fourth, all participants received elixir from a single batch containing verified extract; batch-to-batch variation is unknown. Fifth, the 30-day duration precludes assessment of long-term safety, sustained efficacy beyond one month, or discontinuation effects. Sixth, recruitment through social media and health food stores may have selected for individuals interested in natural products, limiting generalizability.

Seventh, participants had self-reported sleep difficulties but not formally diagnosed insomnia disorder; results may differ in clinical populations. Eighth, effects on sleep architecture (REM sleep, slow-wave sleep) remain unknown without polysomnography. Ninth, the study was conducted by the product manufacturer (Ahara Mushrooms), which could introduce bias despite third-party verification; independent academic replication is essential. Finally, the proprietary formulation limits full reproducibility, though COA verification of adenine content provides a reproducible target for other researchers.

5. CONCLUSIONS

This study provides preliminary evidence that sublingual Reishi mushroom elixir with third-party verified adenine and adenosine content in the extract, delivered via nanofiber technology, significantly improves sleep quality in adults with chronic sleep difficulties. Key findings include 85% clinically meaningful improvement, 66% reduction in sleep onset latency, no tolerance development, excellent safety profile, and high participant satisfaction (95% continuation intent, 100% willing to recommend).

The combination of verified adenine/adenosine content, nanofiber delivery technology for enhanced bioavailability, and sublingual administration bypassing digestive degradation may explain the robust effects achieved with this intervention. These preliminary findings suggest adenine-containing Reishi elixir represents a promising non-hormonal sleep support approach that works with the body's natural adenosine sleep-regulation system. The absence of tolerance—a major limitation of existing sleep aids—is particularly noteworthy if confirmed in longer studies.

Critical next steps: Placebo-controlled randomized trials with objective sleep measurement (polysomnography) are essential to confirm these preliminary findings. Longer-duration studies (6-12 months) are needed to assess long-term safety, sustained efficacy, and potential late-onset tolerance. Mechanistic studies characterizing pharmacokinetics and receptor interactions would clarify the biological basis of observed effects.

For consumers seeking natural alternatives to melatonin and sleeping pills, products with verified adenine content and advanced delivery technology may offer advantages in efficacy, tolerability, and sustained effectiveness. However, prospective users should be counseled that effects typically develop over the first week rather than providing immediate sedation.

This research establishes a foundation for further investigation of adenine-containing botanical elixirs as a novel class of natural sleep interventions targeting the brain's endogenous adenosine system.

AUTHOR CONTRIBUTIONS

Ahara Research Team: Study design, data collection, data analysis, manuscript preparation.

FUNDING STATEMENT

This research was internally funded by Ahara Mushrooms. No external grants or funding sources were received.

COMPETING INTERESTS STATEMENT

This study was conducted by Ahara Mushrooms, the manufacturer of the Reishi elixir evaluated. The authors are affiliated with Ahara Mushrooms and have a financial interest in the outcomes of this research.

To mitigate potential bias, the extract component was analyzed by an independent, ANAB-accredited third-party laboratory (Altitude Consulting) using standardized HPLC methodology. The Certificate of Analysis provides objective verification of extract composition independent of company claims.

Despite these safeguards, readers should consider the potential for bias inherent in manufacturer-sponsored research. Independent academic replication of these findings by researchers without financial interests in the outcome is essential to establish definitive efficacy.

ETHICS STATEMENT

This study was conducted in accordance with ethical research standards for observational evaluations of commercially available dietary supplements. As an observational study of a non-investigational product in adults, formal Institutional Review Board (IRB) approval was not required per 45 CFR 46.104(d). All participants provided informed consent and were free to withdraw at any time. Participant confidentiality was maintained throughout the study.

DATA AVAILABILITY STATEMENT

Anonymized participant data (sleep logs, PSQI scores, exit survey responses) and the Certificate of Analysis (COA) for the extract batch used in this study (Lab Number: 24110127-2, Altitude Consulting) are available upon reasonable request to the corresponding author at research@aharamushrooms.com. Proprietary formulation details are not disclosed but do not prevent replication of key findings (adenine content, delivery method, and clinical outcomes).

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REFERENCES

1. Institute of Medicine (US) Committee on Sleep Medicine and Research. Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem. Washington (DC): National Academies Press; 2006.

2. Cappuccio FP, Cooper D, D'Elia L, Strazzullo P, Miller MA. Sleep duration predicts cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. Eur Heart J. 2011;32(12):1484-1492.

3. Luyster FS, Strollo PJ Jr, Zee PC, Walsh JK. Sleep: a health imperative. Sleep. 2012;35(6):727-734.

4. Winkelman JW. Insomnia Disorder. N Engl J Med. 2015;373(15):1437-1444.

5. Morin CM, Jarrin DC. Epidemiology of Insomnia. Sleep Med Clin. 2022;17(2):173-191.

6. Wachtel-Galor S, Yuen J, Buswell JA, Benzie IFF. Ganoderma lucidum (Lingzhi or Reishi): A Medicinal Mushroom. In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; 2011.

7. Sanodiya BS, Thakur GS, Baghel RK, Prasad GB, Bisen PS. Ganoderma lucidum: a potent pharmacological macrofungus. Curr Pharm Biotechnol. 2009;10(8):717-742.

8. Xu Z, Chen X, Zhong Z, Chen L, Wang Y. Ganoderma lucidum polysaccharides: immunomodulation and potential anti-tumor activities. Am J Chin Med. 2011;39(1):15-27.

9. Paterson RR. Ganoderma - a therapeutic fungal biofactory. Phytochemistry. 2006;67(18):1985-2001.

10. Ebrahim IO, Shapiro CM, Williams AJ, Fenwick PB. Alcohol and sleep I: effects on normal sleep. Alcohol Clin Exp Res. 2013;37(4):539-549.

11. Roehrs T, Roth T. Sleep, sleepiness, and alcohol use. Alcohol Res Health. 2001;25(2):101-109.

12. Borbély AA, Daan S, Wirz-Justice A, Deboer T. The two-process model of sleep regulation: a reappraisal. J Sleep Res. 2016;25(2):131-143.

13. Porkka-Heiskanen T, Strecker RE, McCarley RW. Brain site-specificity of extracellular adenosine concentration changes during sleep deprivation and spontaneous sleep. Neuroscience. 2000;99(3):507-517.

14. Basheer R, Strecker RE, Thakkar MM, McCarley RW. Adenosine and sleep-wake regulation. Prog Neurobiol. 2004;73(6):379-396.

15. Huang ZL, Qu WM, Eguchi N, et al. Adenosine A2A, but not A1, receptors mediate the arousal effect of caffeine. Nat Neurosci. 2005;8(7):858-859.

16. Scammell TE, Arrigoni E, Lipton JO. Neural circuitry of wakefulness and sleep. Neuron. 2017;93(4):747-765.

17. Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev. 1999;51(1):83-133.

18. Henderson JF, Paterson AR. Nucleotide Metabolism: An Introduction. Academic Press; 1973.

19. Moffatt BA, Ashihara H. Purine and pyrimidine nucleotide synthesis and metabolism. Arabidopsis Book. 2002;1:e0018.

20. Smart JD. Buccal drug delivery. Expert Opin Drug Deliv. 2005;2(3):507-517.

21. Patel VF, Liu F, Brown MB. Advances in oral transmucosal drug delivery. J Control Release. 2011;153(2):106-116.

22. Zhang H, Zhang J, Streisand JB. Oral mucosal drug delivery: clinical pharmacokinetics and therapeutic applications. Clin Pharmacokinet. 2002;41(9):661-680.

23. Shojaei AH. Buccal mucosa as a route for systemic drug delivery: a review. J Pharm Pharm Sci. 1998;1(1):15-30.

24. Kulkarni A, Suzuki Y, Sahoo SK. A review on nanofibrous membranes for drug delivery applications. Curr Drug Deliv. 2016;13(6):901-913.

25. Hu X, Liu S, Zhou G, Huang Y, Xie Z, Jing X. Electrospinning of polymeric nanofibers for drug delivery applications. J Control Release. 2014;185:12-21.

26. Stasiak M, Röben E, Rosenboom JG, et al. Electrospun nanofibers for sublingual drug delivery. Drug Deliv Transl Res. 2019;9(6):1156-1166.

27. Verreck G, Chun I, Rosenblatt J, et al. Incorporation of drugs in an amorphous state into electrospun nanofibers composed of a water-insoluble, nonbiodegradable polymer. J Control Release. 2003;92(3):349-360.

28. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28(2):193-213.

29. Backhaus J, Junghanns K, Broocks A, Riemann D, Hohagen F. Test-retest reliability and validity of the Pittsburgh Sleep Quality Index in primary insomnia. J Psychosom Res. 2002;53(3):737-740.

30. Hughes CM, McCullough CA, Bradbury I, et al. Acupuncture and reflexology for insomnia: a feasibility study. Acupunct Med. 2009;27(4):163-168.

31. Walker MP. The role of sleep in cognition and emotion. Ann N Y Acad Sci. 2009;1156:168-197.

32. Pilcher JJ, Huffcutt AI. Effects of sleep deprivation on performance: a meta-analysis. Sleep. 1996;19(4):318-326.

33. Gringras P, Nir T, Breddy J, Frydman-Marom A, Findling RL. Efficacy and safety of pediatric prolonged-release melatonin for insomnia in children with autism spectrum disorder. J Am Acad Child Adolesc Psychiatry. 2017;56(11):948-957.

34. Lader M. Benzodiazepines revisited--will we ever learn? Addiction. 2011;106(12):2086-2109.

35. Schredl M, Berger M, Riemann D. The effect of trimipramine on dream recall and dream emotions in depressive outpatients. Psychiatry Res. 2009;167(3):279-286.

36. Pagel JF, Helfter P. Drug induced nightmares - an etiology based review. Hum Psychopharmacol. 2003;18(1):59-67.

37. Dijk DJ, Archer SN. Light, sleep, and circadian rhythms: together again. PLoS Biol. 2009;7(6):e1000145.

38. Landolt HP. Sleep homeostasis: a role for adenosine in humans? Biochem Pharmacol. 2008;75(11):2070-2079.

39. Xie L, Kang H, Xu Q, et al. Sleep drives metabolite clearance from the adult brain. Science. 2013;342(6156):373-377.

40. Allison C, Pratt JA. Neuroadaptive processes in GABAergic and glutamatergic systems in benzodiazepine dependence. Pharmacol Ther. 2003;98(2):171-195.

41. Dubocovich ML, Markowska M. Functional MT1 and MT2 melatonin receptors in mammals. Endocrine. 2005;27(2):101-110.

42. Landolt HP, Dijk DJ, Gaus SE, Borbély AA. Caffeine reduces low-frequency delta activity in the human sleep EEG. Neuropsychopharmacology. 1995;12(3):229-238.

43. Bjorness TE, Greene RW. Adenosine and sleep. Curr Neuropharmacol. 2009;7(3):238-245.

44. Ferracioli-Oda E, Qawasmi A, Bloch MH. Meta-analysis: melatonin for the treatment of primary sleep disorders. PLoS One. 2013;8(5):e63773.

45. Brzezinski A, Vangel MG, Wurtman RJ, et al. Effects of exogenous melatonin on sleep: a meta-analysis. Sleep Med Rev. 2005;9(1):41-50.

46. Foldvary-Schaefer N, Klem G, Hammerstad J, Frost J, DeBiase A, Dinner DS. The effects of benzodiazepines on the sleep of patients with epilepsy. Clin EEG Neurosci. 2002;33(2):65-68.

47. Vermeeren A. Residual effects of hypnotics: epidemiology and clinical implications. CNS Drugs. 2004;18(5):297-328.

48. Allison C, Pratt JA. Neuroadaptive processes in GABAergic and glutamatergic systems in benzodiazepine dependence. Pharmacol Ther. 2003;98(2):171-195.

49. Dubocovich ML, Hudson RL, Sumaya IC, Masana MI, Manna E. Effect of MT1 melatonin receptor deletion on melatonin-mediated phase shift of circadian rhythms in the C57BL/6 mouse. J Pineal Res. 2005;39(2):113-120.

50. Kunz D, Mahlberg R, Müller C, Tilmann A, Bes F. Melatonin in patients with reduced REM sleep duration: two randomized controlled trials. J Clin Endocrinol Metab. 2004;89(1):128-134.

51. Lemoine P, Zisapel N. Prolonged-release formulation of melatonin (Circadin) for the treatment of insomnia. Expert Opin Pharmacother. 2012;13(6):895-905.

52. Wachtel-Galor S, Tomlinson B, Benzie IF. Ganoderma lucidum ("Lingzhi"), a Chinese medicinal mushroom: biomarker responses in a controlled human supplementation study. Br J Nutr. 2004;91(2):263-269.

53. Yuen JW, Gohel MD. Anticancer effects of Ganoderma lucidum: a review of scientific evidence. Nutr Cancer. 2005;53(1):11-17.

54. Sateia MJ, Buysse DJ, Krystal AD, Neubauer DN, Heald JL. Clinical practice guideline for the pharmacologic treatment of chronic insomnia in adults: an American Academy of Sleep Medicine clinical practice guideline. J Clin Sleep Med. 2017;13(2):307-349.

55. Mitchell MD, Gehrman P, Perlis M, Umscheid CA. Comparative effectiveness of cognitive behavioral therapy for insomnia: a systematic review. BMC Fam Pract. 2012;13:40.

56. Riemann D, Perlis ML. The treatments of chronic insomnia: a review of benzodiazepine receptor agonists and psychological and behavioral therapies. Sleep Med Rev. 2009;13(3):205-214.

57. Haynes RB, Ackloo E, Sahota N, McDonald HP, Yao X. Interventions for enhancing medication adherence. Cochrane Database Syst Rev. 2008;(2):CD000011.

58. Buscemi N, Vandermeer B, Hooton N, et al. The efficacy and safety of exogenous melatonin for primary sleep disorders. A meta-analysis. J Gen Intern Med. 2005;20(12):1151-1158.

59. Perlis ML, Grandner MA, Brown GK, et al. Nocturnal wakefulness as a previously unrecognized risk factor for suicide. J Clin Psychiatry. 2016;77(6):e726-e733.