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Short communication

The effects of *Hericium erinaceus* (Amyloban® 3399) on sleep quality and subjective well-being among female undergraduate students: A pilot study

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1. Introduction

Currently in Japan, one out of every four to five people suffers from sleep disorders [1,2]. A very high percentage of undergraduate students, who are at the last stage of adolescence, suffer from sleep problems because of staying up late at night and sleeping until late in the morning or maintaining irregular sleep patterns. These behaviors lead to disruption of circadian rhythms and deterioration of quality of life, involving a decline in productivity due to daytime sleepiness [3].

Compared with students who get adequate sleep (6–8 h per night), students who habitually sleep in excess (more than 9 h per night) or inadequately (less than 5 h per night) have a strong self-awareness of emotional and physical distress as measured by General Health Questionnaire (GHQ-28). In particular, the levels of salivary free 3-methoxy-4-hydroxyphenylglycol (free-MHPG) (a metabolite of central noradrenaline [NA]), and immunoglobulin A (s-IgA) antibodies (which play a role in the immune system), are substantially lower among students with excessive sleep. Our previous study indicated that disturbance of sleep habits is closely related to decline in subjective well-being, as well as a weakening of immune functions and NA systems [4].

Hericium erinaceus (Lion's Mane mushroom) has long been used for culinary and medicinal purposes. Its cognitive benefits have recently drawn more attention, and studies have investigated the therapeutic use of this mushroom in patients with mild dementia [5–7]. In addition, Nagano et al. [8] reported that after 4 weeks of *H. erinaceus* administration, participants' feelings of depression, irritability, and fatigue significantly declined compared with those before administration, suggesting its efficacy in improving mood. These findings suggest that *H. erinaceus* may be beneficial for people who suffer from circadian rhythm disorders and help alleviate sleep problems, while improving the quality of life for people who lead an unhealthy or non-productive lifestyle by habitually staying up late and sleeping in.

This pilot study evaluated the effects of a 4-week administration of *H. erinaceus* (Amyloban® 3399) on female undergraduate students who were likely to have a high incidence of sleep problems. We assessed changes in sleep quality and subjective well-being with the GHQ-28 and PSQI (Pittsburg Sleep Quality Index). Furthermore, we examined the level of salivary free-MHPG after awakening, which is generally regarded as an accurate index of chronic stress and depressive symptoms and reflects sympathetic nervous system activity [9]. Thus, we were able to conduct a comprehensive analysis of the effects of Amyloban® 3399 on sleep quality and subjective well-being of female students.

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2. Material and methods

2.1. Participants

The participants were eight female undergraduate students (mean age: 21.7 ± 0.4 years), all of whom had a National Dietitian Exam scheduled within 1 month. None of the participants had a history of serious illnesses or prescription medicine use.

2.2. Procedure

The participants were asked to visit the lab and were given a brief description of the experimental procedures and saliva collection method. A questionnaire sheet consisting of GHQ-28 and PSQI questions was distributed. In addition to completing the questionnaires, the participants were asked to fill out their grade level, gender and age.

2.3. Measurements

2.3.1. Questionnaires

GHQ-28 [10]: This questionnaire assessed the participants' mental health over the past week. This self-administered questionnaire consisted of 28 items, divided into four subscales: "somatic symptoms," "anxiety and insomnia," "social dysfunction," and "depression." Participants responded to the questions using a four-point scoring system. Each subscale had a seven-point maximum. Cut-off points were the following: 2–3 points is a mild, and 4 points and higher is considered a moderate for "somatic symptoms" and "anxiety and insomnia,", 1–2 point score is mild, and 3 points and higher is considered moderate symptom for "social dysfunction" and "depression".

PSQI [11]: This questionnaire was used to assess sleep habits and sleep quality over the 1-month period. There were 18 items containing seven components (quality of sleep, sleep duration, sleep latency, sleep efficiency, sleep disturbance, use of sleep medication, and excessive daytime sleepiness). Higher scores suggested poor sleep quality, with a score of 5.5 being the cut-off point.

2.3.2. Saliva collection and free-MHPG measurement

Each participant was asked to collect saliva samples immediately upon waking. A Spitz device ("Sarisoft") was used to collect the samples, which were immediately stored in a −80 °C freezer. All samples were collected at the end of the study. The free-MHPG level was measured using gas chromatography mass spectrometry (Hitachi-M80B, Hitachi, Japan), as described by Yajima et al. [12].

2.4. Administration of H. erinaceus

Participants in this study self-administered six tablets per day of Amyloban® 3399 (Mushroom Wisdom, Inc., East Rutherford, NJ USA), divided into 2 or 3 doses. The supplement was taken with food for a period of 4 weeks. Participants were not explained anything about Amyloban® 3399 during registration; they were just told that "This is a kind of supplement."

2.5. Ethical considerations

The ethics committee of the university approved this study. Participants' safety was the priority and the research data were used for the purposes of this study only. Participants' information was kept confidential. They were given written and oral explanations before providing their consent.

2.6. Statistical analysis

Data analysis was performed with a Windows version of SPSS (Statistical Package for the Social Sciences). A *t*-test was used to evaluate the mean difference between GHQ-28 subscale scores, PSQI scores, and average level of salivary free-MHPG before and after administration of Amyloban® 3399. In each statistical analysis, a *p*-value less than 0.05 was considered statistically significant, and a *p*-value less than 0.10 was considered a marginally significant difference.

3. Results

The average PSQI score before administration of Amyloban® 3399 was 7.3. Six of the participants scored higher than the cut-off point (5.5) and two scored below. The scores (mean ± standard deviation) for the GHQ-28 subscales were as follows: "somatic symptoms," 3.8 ± 2.7 (mild), "anxiety and insomnia," 5 ± 1.9 (moderate), "social dysfunction," 1.9 ± 2.1 (mild), and "depression," 1.8 ± 1.3 (mild).

On the "anxiety and insomnia" subscale of the GHQ-28, there was a declining trend after 4 weeks of supplement administration ($t = 1.86$, $df = 14$, $p < 0.10$). No significant differences were observed on the other subscales after the 4-week administration of Amyloban® 3399. There were no statistically significant differences in PSQI scores associated with Amyloban® 3399 use. However, after the 4-week administration, the average score showed a decline (pre-administration: 7.25, post-administration: 5.75), and the number of participants who scored above the 5.5 cut off point declined from six to four. After 4 weeks of Amyloban® 3399 administration, levels of salivary free-MHPG significantly increased compared with those during pre-administration ($t = -2.25$, $df = 14$, $p < 0.05$; Table 1).

4. Discussion

This study comprehensively evaluated subjective ratings on the GHQ-28 and PSQI questionnaires, as well as the objective assessment of salivary free-MHPG levels, taken from 8 female undergraduate students to assess the effects of 4 weeks of administration of Amyloban® 3399 on sleep quality and subjective well-being. The average PSQI score from the eight participants before administrating Amyloban® 3399 was 7.3 and was higher than the cut-off point (5.5). In addition, the average score on the "anxiety and insomnia" subscale of the GHQ-28 questionnaire before the administration was 5 points, and the percentage of those exhibiting moderate symptoms was high as well. These results reflect a disturbance in sleep habits, and an increase in negative mood and anxiety levels associated with preparations for the national exam that all the participants were scheduled to take in about a month.

Table 1
Comparison of before and after *Hericium erinaceum* intake.

	Before *Hericium erinaceum*	After *Hericium erinaceum*	*p* value
GHQ-28			
Somatic symptoms	3.8 ± 2.7	3.4 ± 1.7	$p = 0.745$
Anxiety and insomnia	5 ± 1.9	3.3 ± 1.8	$p = 0.084$
Social dysfunction	1.9 ± 2.1	1.9 ± 1.5	$p = 1.000$
Depression	1.8 ± 1.3	1.0 ± 1.6	$p = 0.319$
PSQI	7.3 ± 2.6	5.8 ± 2.9	$p = 0.292$
Salivary free-MHPG	5.6 ± 1.9	9.5 ± 4.0	$p = 0.029$

Values are represented as mean ± standard error.
The *p* values indicate the difference between before and after *Hericium erinaceum* intake.

After 4 weeks of Amyloban® 3399 use, the "anxiety and insomnia" score decreased. PSQI scores also decreased, although this difference was not statistically significant. Inanaga [13] has reported that intake of Amyloban® 3399 improves negative mood such as irritability and anxiety, and raises incentive associated with improved concentration and motivation. In addition, he also reported that Amyloban® 3399 was effective in improving symptoms of sleep apnea [13]. These findings suggest that taking *H. erinaceus* (Amyloban® 3399) could improve negative mood and sleep disorder symptoms. However, 2 out of 8 participants in this study did not have sleep problems as assessed by PSQI. Therefore, this study may have lacked the statistical power necessary to examine the effects of Amyloban® 3399 on sleep disturbances. Further studies will be required to investigate the effects of Amyloban® 3399 on circadian rhythm sleep disorders and/or sleep disturbance using a larger number of participants.

In this study, the level of salivary free-MHPG before administration of Amyloban® 3399 was 5.6 ± 1.9 ng/ml. This was low in comparison with the levels from healthy participants in our previous studies (9.3 ± 1.8 ng/ml) [4]. However, after 4 weeks of Amyloban® 3399 use, these levels increased to levels comparable with those in healthy participants. It is suggested that the levels of salivary free-MHPG in people with unidentified complaints are low immediately after awakening. The possible correlation between changes in salivary levels of free-MHPG after awakening and subjective stress reaction needs to be investigated in further detail. Shimbo et al. [14] reported that Erinacine A, which is isolated from *Hericium erinaceum*, enhanced the synthesis of nerve growth factor (NGF) by increasing the secretion of NA and catecholamines. Our study was consistent with these findings with regard to the increase in salivary free-MHPG. Furthermore, an increase in salivary free-MHPG levels coincided with a trend towards improvement in anxiety levels and sleep quality. These results indicated that one of the possible effects of Amyloban® 3399 could be an ability to balance out the mind and body. Thus, the administration of *H. erinaceus* (Amyloban® 3399) could improve mood and circadian rhythm sleep disorder symptoms, as well as the quality of life, in unhealthy people ("semi-healthy" people at early stages of illness) who led an unproductive lifestyle. However, salivary free-MHPG level is known to be related to depressive mood and anxiety. Increased levels of free-MHPG in conjunction with increased psychological stress have also been reported [15,16]. Therefore, further studies would be required to elucidate the impact of Amyloban® 3399 on salivary free-MHPG levels.

5. Conclusion

This pilot study assessed the effects of 4 weeks of administration of Amyloban® 3399 on subjective well-being and sleep quality in female undergraduate students. The results revealed an increase in salivary free-MHPG, which corresponded to an improvement in anxiety and quality of sleep. Thus, we conclude that one of the possible effects of Amyloban® 3399 is to balance out the mind and body. In the future, we will need to study the effects of Amyloban® 3399 on sleep quality and everyday work, using a larger number of student participants.

Footnotes

Amyloban® 3399 made based on a proprietary extract called "Amycenone" was used for this study. It contains standardized amounts of the following compounds:

1. Hericenone (0.5%) – Hericenone stimulates synthesis of nerve growth factor, which promotes nerve protection [17,18].
2. Amyloban (6%) – Fat soluble compound, which reduces the endoplasmic reticulum stress caused by amyloid beta and helps increase the survival of nerve cells [7].

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