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EFFICACY OF NATURAL TRYPTOPHAN FROM LOTUS SEEDS ON SLEEP QUALITY AND OTHER SLEEP-RELATED PARAMETERS

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ABSTRACT

This study aimed to evaluate the efficacy of lotus seeds consumption on sleep quality and other sleep-related parameters. Thirteen participants consumed 80 g of roasted lotus seeds that contained approximately 250 mg of tryptophan daily for 4 weeks. Subjective sleep measurement (Thai version of the Pittsburgh Sleep Quality Index (Thai-PSQI)), Daily Sleep Diary, Stress Test Questionnaire (ST5), the Revised Piper Fatigue Scale (RPFS) and nutritional assessment including anthropometric and dietary assessments were tools for the evaluation. Daily 80 g of roasted lotus seeds consumption for 4 weeks significantly improved sleep quality (6.2 ± 2.5), habitual sleep efficiency (92.9 ± 5.8 %), total sleep time (6.8 ± 0.8 h), sleep onset latency (36.9 ± 13.1 min) and stress (5.7 ± 3.4). Moreover, day-time dysfunction, feeling refreshed after waking up, and fatigue also improved. The study recommends that consumption of roasted lotus seeds, which contain natural tryptophan, is beneficial to sleep and can potentially relieve insomnia.

Keywords: lotus seeds, tryptophan, sleep quality

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Introduction

Poor sleep quality may have negative effects on daily activities, such as impaired performance, low attention, irritability, fatigue, stress, and decreased social interaction.[1] Sleep deprivation is common especially among university students, and has been associated with poor academic performance, mood swing, lack of motivation and physical dysfunction.[2] University student were reported poor sleep quality than any other groups of population.[3]

Tryptophan is an essential amino acid that is derived exclusively from foods. It is metabolized to serotonin and then to melatonin, which promotes sleep and mental health.[4] The essential amino acid tryptophan is the precursor of serotonin. High tryptophan levels in the brain induce the synthesis of serotonin. Plasma tryptophan crossing the blood-brain barrier is preferred by a higher plasma concentration of tryptophan in comparison with other competing large neutral amino acids (LNAAs). The higher plasma tryptophan/large neutral amino acids ratio is affected by the sufficiency of dietary carbohydrates.[5,6] Therefore, vitamin B3 (niacin) promotes the availability of tryptophan for serotonin synthesis. The role of vitamin B3 suppresses the activity of tryptophan 2,3-dioxygenase, which is a key enzyme in the conversion of tryptophan to nicotinic acid (niacin). As a result, tryptophan is converted to niacin, it will not be available as a precursor to serotonin.[5] The recommended tryptophan intake should be from a natural source and the recommended daily intake for adults is estimated to be not more than 250 mg/day to maintain nitrogen balance (proxy measure of protein balance) and avoid side effects of pharmaceutical tryptophan such as nausea, vomiting, headache and dizziness etc.[7]

Lotus (*Nelumbo nucifera* Gaertn.) is also known as water lily. *Nelumbinis semen* is commonly called as lotus seeds that have been used as a vegetable, functional food, and traditional medicine for 7,000 years ago.[8] Seeds of the lotus plant are rich source of nutrients and bioactive compounds. Macronutrients consist of approximately 70% of carbohydrates, 21% of total protein and 3% of lipid. Lotus seeds are high in tryptophan content (326 mg tryptophan /100 g of lotus seed.[8] In addition to macronutrients, they also contain a high quantity of minerals, such as phosphorus, calcium, magnesium, and some vitamin B. The lotus seeds play a vital role in traditional medicine practices, these seeds also be used as roasted, raw, boiled, and dry powder.[9]

The lotus seed is used in the treatment of insomnia. Recent studies have shown that lotus seeds promoted sleep through the GABAergic system in the *Drosophila melanogaster* model.[10] In addition, neuropharmacological activity of ethanol extract of *Nelumbo nucifera* Gaertn. seeds increase sleep time in rats.[11]

In human studies, protein source tryptophan intake of 250 mg with carbohydrates at night for a week improved sleep quality[12] and tryptophan intake at breakfast combined with daytime light exposure on salivary melatonin secretion at night which seems to decrease sleep onset.[13]

The American Heart Association recommends up to 6 portion size of dried seeds per day. One portion size estimated 40 g or one handful of dried seeds.[14]

Lotus seeds are proper food for sleep promotion because they consist of carbohydrate, tryptophan and niacin, which play roles in the synthesis of serotonin. This study aimed to evaluate the efficacy of lotus seed consumption on sleep quality and other sleep-related parameters that could be improved through nutritional therapy by proportioning lotus seed consumption.

Methods

This study utilized a free-living, self-controlled design. Participants were asked to maintain stable dietary and life activities throughout the study for 4 weeks. Ethical approval was obtained from Human Research Committee of Valaya Alongkorn Rajabhat

University under the Royal Patronage, Pathumthani, Thailand. (Rec.N0 42/2563)

Participants and recruitment

The participants were students of Valaya Alongkorn Rajabhat University under the Royal Patronage who had poor sleep quality. The study was conducted during the non-examination period during March 2021 – May 2021.

Inclusion Criteria

Participants between the ages of 20 - 30 years old were recruited for the study. Eligible participants who had poor sleep quality were assessed by the Thai version of the Pittsburgh Sleep Quality Index (Thai-PSQI) and the Thai-PSQI score of ≥ 5 points was used as a cut-off point indicating poor sleep.[15] All participants did not use hypnotic drugs and/or sedative supplement products. Participants also did not have a history or present illnesses such as liver disease, kidney disease, thyroid disease or infectious diseases.

Exclusion Criteria

Participants were excluded from the study if they had mental health illnesses, metabolic disorders, or food allergies. They were also excluded if they were pregnant, shift worker, using medications for the treatment of insomnia or mental health disorders, unwillingness to participate during the study or complete the questionnaire.

Sample size calculation

The sample size for the study was calculated using the effect size formula according to Lin H-H *et al.*[16] and interpretation by Cohen[17] at the power of 0.80 (β), and statistically significant of 0.05 (α). Ten participants were required and additional 30% of the sample size was recruited to compensate for drop-outs. Therefore, a total sample size of 13 participants was recruited.

Diet Description

Participants were given 80 g of roasted lotus seeds *(Nelumbo nucifera* Gaertn.*)*. The nutritional value of 80 g of lotus seeds contained tryptophan 253 mg, energy 282 kcal, carbohydrate 51 g (72.3% of total energy), protein 15 g (21.3% of total energy), fat 1.7 g (5.4% of total energy), niacin 1.4 mg and fiber 8.1 g. The database was reviewed in the Bureau of Nutrition, Department of Health, Ministry of Public Health.[18]

Clinical Study Process

The participants completed the 6-week study, which was divided into 3 study periods: baseline period (2 weeks before the study), intervention period (4 weeks), and last period (2 weeks post lotus seeds consumption). At the baseline (week 0), participants were assessed sleep quality, other sleep-related parameters and nutritional parameters for 2 weeks before the study. During the intervention period (weeks 1-4), participants consumed roasted lotus seeds of 40 g, twice a day, i.e. 1.5 h before breakfast and 1.5 h before bedtime, which was equivalent to approximately 250 mg of tryptophan per day. The sleep quality, other sleep-related parameters and nutritional parameters were assessed. Participants were continued to be assessed in the last period (week 5-6).

Instruments and Measurements

Participants were measured the following: sleep quality, other sleep-related parameters and nutritional parameters at weeks 0, 4 and 6. The instruments that were in the study are:

1. Subjective measurement, which were assessed to sleep quality and other variable related sleep parameters.

1.1 Sleep quality was evaluated by Thai-PSQI. Thai-PSQI is considered a gold standard for the measurement of subjective sleep quality. It is a valid and reliable tool for screening and identifying the presence of significant sleep disturbances.[15] A self-scored questionnaire was used to assess subjective sleep quality and sleep disturbance in the prior 1 month ago. It generated 7 component scores, the totals score was added to the final score of 0-21 points. Participants with a Thai-PSQI score of ≥ 5 were defined as having poor sleep quality. The total score

of Thai-PSQI classified the level of sleep quality including good sleep quality (scored 0), mild sleep difficulty (scored 1-7), moderate sleep difficulty (scored 8-14), and severe sleep difficulty (scored 15-21).

1.2 Daily Sleep Diary was adapted from Hudson.[12] The diary is a self-recorded sleep information on bedtime, wake up time, sleep duration, sleep onset latency, habitual sleep efficiency (actual sleep time between sleep onset and final awakening /total sleep time x 100), The sleep efficiency of more than 85% was considered good, and feeling refreshed after waking up, which included perception of rejuvenation in the morning by 3-Point Likert Scale as refreshing (3 points), moderately refreshing (2 points), and slightly refreshing or not refreshing (1 points).

2. Stress was evaluated using stress test questionnaire (ST5), which was developed by Department of Mental Health, Ministry of Public Health of Thailand.[19]

A self-scored questionnaire is a rated 4-Point Likert Scale. The total score of ST5 classified the level of stress including no stress (scored 0-4), moderate stress (scored 5-7), high stress (scored 8-9), and severe stress (scored $\geq$ 10).

3. Fatigue was evaluated using the Revised Piper Fatigue Scale (RPFS),[20] a self-scored questionnaire of fatigue as a numeric rating scale. The scale used a rating of 1 (not at all) to 10 (very much). The total score was added to generate the final score of 0-130 points, indicating 3 levels of fatigue as extremely fatigue (93-130 points), moderately fatigue (53-92 points), and no fatigue (13-52 points).

4. Nutritional parameter was assessed using the anthropometric and dietary measurement.[21]

4.1 Anthropometric measurement, body composition as body weight was evaluated by Bioelectrical Impedance Analysis: BIA, Tanita BC-601 Body Composition Analyzer, Tanita Co. Ltd., Japan for initial health information of participants.

4.2 Dietary measurement was evaluated using a self-reported 24-hour dietary recall method to assess energy and nutrient intakes during the study. There were analyzed by the INMUCAL-Nutrients version 3 software developed by the Institute of Nutrition, Mahidol University.[22]

5. Compliance and safety of roasted lotus seed consumption was evaluated using questionnaires. During weeks 1-4, participants had a follow-up visit to receive roasted lotus seeds. The compliance was checked by questionnaire and they were asked to return any unconsumed roasted lotus seeds and monitored side effects at each visit.

Statistical analysis

Descriptive statistics e.g. percentage, mean, and standard deviation (SD) were used to describe the results. Inferential statistics using repeated measures ANOVA was used to analyze changes in sleep quality, other sleep-related parameters and dietary intake in the study.

Results

Thirteen eligible participants, 2 males and 11 females with the average age of 20.8 ± 0.7 years participated in this study (Table 1). Most of participants were within normal weight status indicated by WHO criteria BMI 23.9 ± 6.7 kg/m^2. These individuals had poor sleep quality (within moderate sleep difficulty), Thai-PSQI score of 9.5 ± 2.3 and the moderate habitual sleep efficiency of 75.4 ± 9.4% at week 0. The participants were of high stress and moderately fatigued with the scores of 8.4 ± 2.9 and 57.8 ± 21.2, respectively.

The average nutritional intake data were derived from self-reported dietary 24 h recall (Table 2). During weeks 1-4, participants were instructed to consume 80 g of roasted lotus seeds daily (containing approximately 250 mg of natural tryptophan). The compliance with the consumption of the roasted lotus seeds was good. The roasted lotus seeds were consumed on average by 95% of the intended amount, resulting in an estimated mean daily intake of 240 mg of tryptophan. There was no report of side effects associated with lotus seeds during the

intervention period. According to the average nutritional intake, there were no significant comparative changes in energy intake, carbohydrate, and fat while the intake of protein and niacin increased significantly between baseline and week 4. In addition, there was also significant increase in tryptophan intake (1167.6 ± 391.3 mg), consisting of tryptophan from dietary intake (919.8 ± 380.6 mg) and roasted lotus seeds (240 ± 12.8 mg). There was no significant change in tryptophan from regular dietary intake over the study period. After quitting roasted lotus seeds consumption for 2 weeks, niacin intake decreased significantly from week 4.

The sleep quality, according to the Thai-PSQI, after 4 weeks of roasted lotus seeds consumption resulted in a significantly lower T-PSQI score from baseline (from 9.5±2.3 at week 0 to 6.2±2.5 at week 4, Table 3). The sleep onset latency significantly reduced (57.7±1.1 min at week 0 and 36.9±13.1 min at week 4) and stress level was also decreased (8.4±2.9 at week 0 and 5.7±3.4 week 4). The habitual sleep efficiency (75.4 ± 9.4% at week 0 and 92.9 ± 5.8% at week 4) and total sleep time (6.2 ± 0.9 h at week 0 and 6.8 ± 0.8 h at week 4) significantly increased after 4 weeks of intervention period. Day-time dysfunction, feeling refreshed after waking up and fatigue improved compared to baseline although not statistically significant. There were no significant comparative changes in Thai-PSQI, habitual sleep efficiency, total sleep time, sleep onset latency and stress at week 6 compared to week 4.

Discussion

The study showed that daily consumption of 80 g of roasted lotus seeds for 4 weeks improved sleep quality, sleep efficiency, total sleep time, sleep onset latency and stress from baseline. Participants were consistent in keeping the dietary diary, which showed the total tryptophan intake of 1167.6 ± 391.3 mg at week 4, which was higher than that in week 0 and week 6 (902.8 ± 324.7 mg, 947.1 ± 223.4 mg respectively). In addition, there was no significant change in tryptophan amount from regular dietary intakes over the study period, indicating the stability of regular dietary intake. The results from this study substantiate the finding of the previous study, which showed that tryptophan (250 mg/d) from de-oiled butternut squash seeds improved sleep quality and sleep efficiency.[12]

Table 1 Characteristics of participants.

Parameters	**Mean ± S.D.**
Gender*	
Male	2 (15)
Female	11 (85)
Age, year	20.8±0.7
Height, cm	162.2±7.2
Weight, kg	62.9±18.7
BMI, kg/m^2	23.9±6.7
Sleep Quality, score	9.5±2.3
Habitual sleep efficiency, %	75.4±9.4
Stress, score	8.4±2.9
Fatigue, score	57.8±21.2

*N (%)

Table 2 Differences in daily energy and nutrient intake during the 6-week study.

Nutritional intakes	Week 0	Week 4	Week 6
Energy, kcal	1494.9±316.5	1578.0±197.3	1379.1±200.7[b]
Carbohydrate, g	207.4±55.0	213.2±33.0	169.0±36.9[b]
Protein, g	59.4±21.4	70.6±13.1[a]	63.0±13.3
Tryptophan, mg	902.8±324.7	1167.6±391.3[a]	947.1±223.4
Sources of tryptophan			
Regular diet, mg	902.8±324.7	919.8±380.6	947.1±223.4
Roasted lotus seed, mg	-	240.0±12.8	-
Fat, g	47.5±16.3	51.1±10.3	53.1±7.6
Niacin, mg	12.9±5.7	15.0±2.8[a]	10.5±2.7[ab]
Vitamin B6, mg	0.41±0.3	0.4±0.3	0.6±0.53
Magnesium, mg	30.4±34.7	41.0±11.2	49.1±29.9
Potassium, mg	1534.5±1277.9	1765.3±256.9	1139.9±301.6[b]
Sodium, mg	2533.9±1383.2	2283.2±508.2	3208.0±1209.4[b]
Drinking water, ml	1458.1±860.2	1294.9±532.2	1200.9±568.0

Significant difference from week 0 [a] $p<0.05$

Significant difference from week 4 [b] $p<0.05$

Table 3 Differences in sleep quality and other sleep-related parameters during the 6-week study.

Parameters	Week 0	Week 4	Week 6
T-PSQI, score	9.5±2.3	6.2±2.5[a]	6.4±2.6[a]
Habitual sleep efficiency, %	75.4±9.4	92.9±5.8[a]	91.8±9.4[a]
Total sleep time, hr	6.2±0.9	6.8±0.8[a]	6.6±0.9
Sleep onset latency, min	57.7±1.1	36.9±13.1[a]	34.6±11.2[a]
Day-time dysfunction, score	5.1±1.9	3.8±2.3	4.8±2.5
Feeling refreshed after waking up, score	1.0±0.7	1.4±0.5	1.2±0.6
Stress, score	8.4±2.9	5.7±3.4[a]	5.5±3.0[a]
Fatigue, score	57.8±21.2	45.0±27.9	56.0±29.3

Significant difference from week 0 [a] $p<0.05$

Significant difference from week 4 [b] $p<0.05$

Previous studies have shown that tryptophan consumption twice a day 90 minutes before breakfast and 90 minutes before bedtime could improve sleep quality[11] and tryptophan intake at breakfast combined with daytime light exposure on salivary melatonin secretion at night and promote quality of sleep.[23] During the intervention period, the amount of niacin increased significantly from baseline. The amount of carbohydrate was higher than week 0 and 6.

After quitting roasted lotus seeds consumption, the amount of carbohydrate and niacin intake reduced significantly from week 4 although all sleep parameters did not significantly change from week 4. Sufficient carbohydrate intake to induce a rapid and sustained increase in serum insulin levels resulted in higher serum tryptophan levels relative to other LNAAs, which allowed tryptophan a competitive advantage for the transport sites to cross the blood-brain barrier for serotonin synthesis. Niacin can be produced endogenously from tryptophan by inhibiting the activity of the 2,3-dioxygenase, with a sufficient amount of tryptophan available to synthesize serotonin.[24,25] The nutritional profile of lotus seeds assessed by proximate analysis shown as the composition of carbohydrate was approximately 70% and most of the essential amino acid was tryptophan.[8] This was the possible mechanism contributing to the sleep-improving effects of roasted lotus seeds. However, the results of this study suggest that further research into the sleep-promoting mechanisms of lotus seeds is necessary.

Interestingly, other parameters including day-time dysfunction, feeling refreshed after waking up, and fatigue were of upward trend in this study. Although the total sleep time (6.8 ± 0.8 hr) and sleep onset latency (36.9±13.1 min) improved during the intervention period, they were still slightly below the recommended values (7-9 hr and < 30 min respectively).[26,27] The Thai-PSQI score also improved from 9.5±2.3 (moderate sleep difficulty) at week 0 to 6.2 ± 2.5 (mild sleep difficulty) and habitual sleep efficiency saw an improvement to above 85%, which is considered good.

Limitations

There are some limitations to consider. The sleep quality was assessed by subjective measurement, which was based on memory and was self-reported. The improvement could be made by implementing objective measurement such as actigraphy. The distribution of gender was female-oriented. This may have an effect on the results of the study as female participants tend to have higher PSQI component scores because of sex hormones.[28]

Conclusion

Daily consumption of 80 g of roasted lotus seeds for 4 weeks improved sleep quality, habitual sleep efficiency, total sleep time, sleep onset latency, stress, and fatigue. The study recommends that the consumption of roasted lotus seeds, which contain natural amino acid tryptophan, are beneficial to sleep and could be used as an alternative relief of insomnia.

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