Sleep-promoting activity of lotus (Nelumbo nucifera) rhizome water extract via GABA_A receptors

Yejin Ahn^a, Singeun Kim^a, Chunwoong Park^a,b, Jung Eun Kim^c, Hyung Joo Suh^a,b and Kyungae Jo^a

^aDepartment of Integrated Biomedical and Life Science, Graduate School, Korea University, Seoul, Republic of Korea; ^bTransdisciplinary Major in Learning Health Systems, Department of Healthcare Sciences, Graduate School, Korea University, Seoul, Republic of Korea; ^cNew Technology Business Team, COSMAXNS, INC, Seongnam, Republic of Korea

ABSTRACT

Context: The sleep-promoting activity of Nelumbo nucifera Gaertn. (Nymphaeaceae) alkaloids in leaves or seeds are well known. However, the sleep-promoting activity of the lotus rhizome (LE), which is used mainly as food, has not yet been evaluated.

Objective: We investigated the sleep-promoting activity of LE water extract.

Materials and methods: Institute of Cancer Research (ICR) mice (n = 8) were subject to a pentobarbital-induced sleep test to assess changes in sleep latency and duration following the administration of LE (80–150 mg/kg). In addition, electroencephalography analysis was performed to detect the sleep quality after LE treatment as well as the sleep recovery effect of LE using a caffeine-induced insomnia SD rat model. Real-time PCR and western blot analysis were performed to investigate the expression of neurotransmitter receptors, and the GABA_A receptor antagonists were used for receptor binding analysis.

Results: An oral administration of 150 mg/kg LE significantly increased sleep duration by 24% compared to the control. Furthermore, LE increased nonrapid eye movement (NREM) sleep by increasing theta and delta powers. In the insomnia model, LE increased sleep time by increasing NREM sleep. Moreover, treatment with picrotoxin and flumazenil decreased the sleep time by 33% and 23%, respectively, indicating an involvement of the GABA_A receptor in the sleep-enhancing activity of LE. The expression of GABA_A receptors and the concentration of GABA in the brain were increased by LE.

Discussion and conclusions: The results suggest that the sleep-promoting activity of LE was via the GABA_A receptor. Collectively, these data show that LE may promote sleep.

Introduction

Humans spend one third of their lives sleeping. Sleep is crucial in human life; during sleep, the brain relieves mental and physical fatigue acquired during work and processes information to strengthen cognitive functions such as memory (Berkley 2021). However, 30%–35% of the world’s population has temporary sleep disorders, and the ratio is particularly high among women and older adults (Ohayon 2011). Sleep disorders are caused by various factors, such as stress, tension, fear and anxiety, and among these, people in modern society tend to experience stress-induced sleep disorders. In fact, 78% of insomnia patients reported that the insomnia was caused by stress (Bastien et al. 2004). Benzodiazepine-based drugs, nonbenzodiazepine-based drugs, benzodiazepine receptor agonists and antidepressants with sedative action have been used as therapeutic drugs (Madari et al. 2021). However, prolonged use of these drugs has side effects, which include resistance and dependence. Therefore, use of alternative drugs that can treat anxiety and insomnia and have fewer side effects is warranted.

Medicinal herbs are the most common alternatives for improving sleep disorders. They have been used for many years, have fewer side effects, and are considered safe. Herbs that have shown to improve sleep disorders through oral administration include ashwagandha [Withania somnifera L Dunal (Solanaceae)], hops [Humulus lupulus L. (Cannabinae)], lemon balm [Melissa officinalis L. (Lamiaceae)], German chamomile [Matricaria recutita L. (Asteraceae)], valerian [Valeriana officinalis L. (Valerianaceae)] and lettuce [Lactuca sativa L. (Asteraceae)] (Kim et al. 2018; Borras et al. 2021; Jo et al. 2021a). Additionally, sleep-promoting and sedative effects of essential oil from leaves of Dysphania ambrosioides L. (Amaranthaceae) (Dougnon and Ito 2021) and Pogostemon cablin Benth. (Lamiaceae) (Ito et al. 2016), respectively, via inhalation, have been reported. The sedative effect of methanol extract of Dorstenia arifolia Lam. (Moraceae) was also confirmed through intraperitoneal injection in a mouse model (Zapat-Sudo et al. 2010). Therefore, these results suggest that herbal medicines can exhibit beneficial effects through various routes such as oral, inhalation and intraperitoneal.

Our previous studies confirmed the sleep-promoting activity of Nelumbo nucifera Gaertn. (lotus) seed and leaf extracts (Jo et al. 2021b; Kim et al. 2021). Interestingly, γ-aminobutyric acid (GABA) present in lotus seeds was found to be the sleep-promoting compound that acted through the GABA_A receptor. Similarly, quercetin-3-glucuronide in lotus leaf extract was found...
to increase sleep time and nonrapid eye movement (NREM) sleep by binding to the GABA<sub>A</sub>-benzodiazepine receptor. Lotus is a perennial aquatic plant that grows in ponds or swamps. In Asian countries, including Korea, China, Japan and Vietnam, all parts of the lotus, including flowers, rhizomes and leaves, are used as food. Along with the lotus leaf, the rhizome has the largest market as an edible food item; it is a common vegetable in China and is used in various dishes or even eaten raw (Yang et al. 2007). In particular, the rhizome has long been used as antipyretic, antidiabetic, hepatoprotective and anti-inflammatory in oriental medicine prescriptions (Mukherjee et al. 1997; Sridhar and Bhat 2007; Zhao et al. 2014). However, to date, the sleep-promoting activity of lotus rhizome (LE) has not been studied. This study investigated the sleep-promoting activity of LEs, which is the second most part consumed after its leaves.

**Materials and methods**

**Sample preparation**

The LE extract prepared by hot water extraction and an enzyme treatment process was obtained from COSMAXNS INC. (Seongnam, Korea). The identity of the plant was ascertained morphologically by Prof. K. S. Shin of Kyonggi University, Suwon, Korea. A voucher specimen (KUCHS-210501) was deposited at the College of Health Sciences, Korea University, Seoul, Korea.

**Experimental animals**

Male Institute of Cancer Research (ICR) mice (5 weeks old, 20 ± 2 g) and male Sprague-Dawley (SD) rats (8 weeks old, 180 ± 20 g) were purchased from Orient Bio Inc. (Seongnam, Korea). They were acclimatized for at least 1 week under the following conditions: temperature 20 ± 2 °C, humidity 50%-5%, 12 h dark/light cycle and ad libitum access to solid food and water. Animal experiments were approved by the Korean University Institutional Animal Care and Use Committee (KUIACUC-2021-0020) and were performed in accordance with approved Animal Care and Use Protocols.

**Measurement of the sleep latency time and duration in pentobarbital-treated mice**

To measure the sleep latency time and duration, mice were subject to fasting for 24 h and then orally administered with LE. After 30 min, 42 mg/kg pentobarbital was injected intraperitoneally. Sleep latency time was defined as the time from intraperitoneal injection of pentobarbital to the loss of the positive reflex, while the recovery time from the loss of the positive reflex was considered as sleep duration. Animals that did not sleep within 15 min of pentobarbital administration were excluded from the experiment (maximally n = 1 per group in the present study). Mice were divided into the following groups (n = 8/group): the negative control (NC) group (saline-administered), positive control (PC) group (benzodiazepine alprazolam, 300 μg/kg) and LE-treated groups (80, 100, 120 and 150 mg/kg).

**Electroencephalography (EEG) analysis in rats**

Electrodes (Emka Technologies, Paris, France) were fixed on the skull surface of SD rats as previously described (Jo et al. 2021b). The LE-administered groups were orally administered with LE 1 h before EEG analysis. EEG changes were recorded between 11:00 and 18:00 every day for 9 days. Alprazolam (300 μg/kg), a sleeping pill, was used as a PC (n = 8/group). Cortical EEGs were obtained using Iox2 (version 2.8.0.13; Emka Technologies) and the spectra were analysed in 1 Hz frequency bins and standard frequency bands (δ = 0.5–4 Hz; θ = 4–8 Hz; α = 8–12 Hz; β = 12–30 Hz; γ = 30–60 Hz). After EEG recording, a Fast Fourier Transform (FFT) was performed every 2 s. Wake (β waves) and sleep times (sleep: sum of REM and NREM; REM: α waves; NREM: sum of δ and θ waves) were calculated using the ecgAUTO3 program (version 3.3.0.20; Emka Technologies) based on average FFT data obtained at 10-s intervals in the 0–30 Hz range (Kim et al. 2021). To assess the sleep-enhancing effect of LE, an insomnia rat model was established by oral administration of caffeine (n = 8/group). In the caffeine control group, caffeine alone was orally administered for 4 days, and in the LE group, LE (120 or 150 mg/kg) was orally administered along with caffeine (40 mg/kg). Next, EEG analysis was performed as described above.

**Analysis of the expression of brain receptors and GABA content in mice**

ICR mice were orally administered with alprazolam or LE for 21 days, and total RNA was extracted from brain cortex tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Real-time quantitative PCR was performed using the SYBR Green<sup>™</sup> kit (Quantitect<sup>™</sup> SYBR Green PCR, Qiagen, Hilden, Germany), and the relative gene expression was calculated using the Ct method (Livak and Schmittgen 2001). The primer types and sequence information used are as follows: GABAA receptor (NM 008073.3; F: GCCCTTGGTGGAGTAGCTGC, R: GCCCTTGAAAGAAAACTCCG), GABA<sub>B</sub> receptor (NM 019439.3; F: TCCGGAACG GGGAAAAGATG, R: TTTACTCGGGACCTTCA) and 5HT<sub>1A</sub> receptor (NM 008314.2; F: CCGATCTCATGGTACATG, R: ACAATCGAGGCGATACAGC).

The GABA content in the brain was analysed by high-performance liquid chromatography (HPLC) as previously described, with some modifications, using a Waters AccQ-Tag column (3.9 × 150 mm<sup>2</sup>; Milford, MA, USA) at 250 nm excitation/395 nm emission wavelengths (Waters 2475 Multi λ Fluorescence Detector) (Jo et al. 2021b). Mobile phases A, B and C were water AccQ-tag Eluent A (acetate-phosphate buffer), acetonitrile and Milli-Q water, respectively.

**Western blot analysis**

After oral administration of alprazolam or LE for 3 weeks, the expression of GABAA<sub>A</sub>, GABA<sub>B1</sub> and 5HT<sub>1A</sub> receptors in the mouse brain cortex was analysed using western blotting. Proteins (50 μg) from mouse brains were separated on 10% Tris-Glycine polyacrylamide gels (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and transferred to Immobilon-P membranes (Millipore Corporation, Bedford, MA, USA). The following primary antibodies were used: anti-GABAA<sub>A</sub> (Abcam, Cambridge, UK; ab72445; 1:1000), anti-GABA<sub>B1</sub> (Cell Signalling Technology, Danvers, MA, USA; #3835; 1:1000) and anti-5HT<sub>1A</sub> (Abcam, ab-85615; 1:1000). Goat anti-rabbit IgG-HRP (Santa Cruz, CA, USA) were employed as the secondary antibody. Visualisation was performed using ECL Prime in the FluorChem E imaging system (Protein Simple, San Jose, CA, USA) using GADPH (Cell Signalling Technology, #5174; 1:1000) as an internal control.
Effects of GABA<sub>A</sub> receptor antagonists on sleep activity in mice

To examine whether the sleep-promoting activity of LE is via GABA<sub>A</sub> receptor binding, sleep latency time and duration were measured in a mouse model using the GABA<sub>A</sub> receptor antagonists picrotixin (PIX) and flumazenil (FLU). For intraperitoneal injection, PIX (4 mg/kg) and FLU (10 mg/kg) were individually dissolved in edible oil (soybean oil), and edible oil alone was intraperitoneally injected in the NC and LE-only (LE) groups. The antagonist was injected 30 min before LE was orally administered to mice. Thereafter, saline was administered to the NC group and the antagonist-only group (PIX, FLU groups). In the LE-administered group, 150 mg/kg of LE, which was the dose showing excellent sleep promoting activity, was orally administered. After 30 min of LE administration, pentobarbital was injected intraperitoneally at a concentration of 42 mg/kg, and the sleep latency time and duration were measured (n = 8/group).

Statistical analysis

Sample size was calculated using software G*Power (version 3.1.9.2; Franz, Universitat Kiel). With a power of 80%, 0.05 level of statistical significance, and effect size of 0.8, the sample size for each test was calculated to be 8. All data are expressed as mean ± standard error of the mean (SEM). Statistical Package for Social Sciences version 12.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis between the experimental groups. A one-way analysis of variance test and Tukey’s post hoc test were performed. Results with p < 0.05 were considered statistically significant.

Results

Effects of LE on sleep latency time and duration of pentobarbital-treated mice

To evaluate the effect of LE on sleep, sleep latency time and duration of pentobarbital-treated mice were measured (Figure 1). Sleep latency was significantly lower in the PC group than in the NC group (p < 0.01). Meanwhile, in the LE-administered groups, there was a decrease in the sleep latency time; the decrease being dose-dependent (80–120 mg/kg). In particular, 100 and 120 mg/kg of LE significantly decreased the sleep latency time in the LE-administered group compared to the NC group (p < 0.05, Figure 1(A)). To the contrary, the sleep latency time in 150 mg/kg LE-administered and NC groups was not significantly different, although the latency time was higher in the 150 mg/kg LE-administered group than in the 120 mg/kg LE-administered group. Furthermore, as expected, the sleep time in the PC group significantly increased compared to that in the NC group (p < 0.01), and LE increased the sleep duration in the LE-administered group in a dose-dependent manner. Compared to the NC group, the sleep duration was longer in the LE-administered group which received 150 mg/kg LE (p < 0.05, Figure 1(B)).

Analysis of the effects of LE on REM and NREM sleep time by EEG

For a more accurate sleep time and quality evaluation, EEG analysis was performed at 120 and 150 mg/kg doses of LE (Figure 2). As seen from the figure, compared to the NC group administered with saline, the wake times were significantly shorter in PC and LE-administered groups (p < 0.001, p < 0.05 and p < 0.01, respectively, Figure 2(A)). To the contrary, there was a significant increase in sleep times of PC and LE-administered groups as compared to those of the NC group (p < 0.001, p < 0.01 and p < 0.01, respectively, Figure 2(B)). The increase in sleep time in the PC group was due to an increase in REM sleep time (p < 0.001, Figure 2(C)), while that in the LE-administered group was due to an increase in NREM sleep time (Figure 2(D)). In particular, in comparison to the NC, theta and delta power of NREM sleep significantly increased in the LE-administration group that received 150 mg/kg LE dose (p < 0.05, Figure 2(E,F)). Collectively, the results showed that the oral administration of LE not only increased NREM, which corresponds to deep sleep, but also increased the sleep time.

Effect on mRNA, protein expression of GABA and serotonin brain receptors and GABA concentration in brain tissue

GABA is a main neurotransmitter in sleep mechanism related to GABA receptors in the hippocampus and frontal lobe. The GABA concentration was significantly higher in benzodiazepine-administered mice than that in the NC group mice (p < 0.001, Figure 3(A)). LE significantly increased the GABA concentration in the brain of LE-administration group compared to the NC group (p < 0.001).

Figure 1. Effects of LE on pentobarbital-induced sleep latency (A) and sleep duration (B) in mice. Mice were orally administered with LE (80, 100, 120 and 150 mg/kg). Data are presented as means ± SEM. *p < 0.05 and **p < 0.01 vs. NC group. NC: negative control group (saline); PC: positive control group (300 μg/kg alprazolam administration); LE: lotus rhizome extract-administered group.

Figure 2. Analysis of the effects of LE on REM and NREM sleep time by EEG. Parameters were measured in the NC, PC and LE groups. REM and NREM sleep times were significantly lower in the PC group than in the NC group (p < 0.05). LE increased the sleep duration in the LE-group in a dose-dependent manner. Compared to the NC group, the sleep duration was longer in the LE-administered group which received 150 mg/kg LE (p < 0.05, Figure 1(B)).

Figure 3. Effects of LE on pentobarbital-induced sleep latency (A) and sleep duration (B) in mice. Mice were orally administered with LE (80, 100, 120 and 150 mg/kg). Data are presented as means ± SEM. *p < 0.05 and **p < 0.01 vs. NC group. NC: negative control group (saline); PC: positive control group (300 μg/kg alprazolam administration); LE: lotus rhizome extract-administered group.
To evaluate the LE related enhancement of sleep mechanism, the level of mRNA and protein expression were analysed after administration of the different doses. To confirm the LE concentration dependence, the experiment was carried out by setting the concentration to 100 and 150 mg/kg more extensively than the previous administration concentrations of 120 and 150 mg/kg. Effect of LE on mRNA expression level of GABA_\text{A}, GABA_\text{B1} and 5HT_1A receptors was measured. It was found that mRNA expression level of GABA_\text{A} receptor was significantly higher in 100 and 150 mg/kg LE-administered groups than in the NC group \((p < 0.01, \text{Figure 3(B)})\). Though the mRNA level of GABA_\text{B1} receptor significantly increased in 150 mg/kg LE group \((p < 0.05)\), the difference was not statistically significant in the group administered 100 mg/kg of LE. Additionally, there was no significant difference in the mRNA expression level of 5HT_1A receptors between the NC and LE-administered groups; however, alprazolam administered (PC) group showed a significantly increased mRNA expression level of serotonin receptor compared to the NC group.

The findings of protein expression on GABA and serotonin receptors were similar to the mRNA expression results. Unlike in the NC group, there was a significant increase in protein expression of GABA_\text{A} and GABA_\text{B1} receptors in the LE-administered groups \((p < 0.001, \text{Figure 3(C)})\).

**Effects of GABA_\text{A} receptor antagonists on the sleep-promoting activity of LE in pentobarbital-induced sleep test**

PIX and FLU were used as GABA_\text{A} receptor antagonists. As compared to LE-administered group, the sleep-promoting activity was reduced in PIX + LE or FLU + LE groups, suggesting that the GABA_\text{A} receptor is involved in sleep-promoting activity of LE (Figure 4).

The results show that the mixture of PIX and LE significantly decreased the sleep latency. The sleep latency time of the LE-administration group receiving an oral dose of 150 mg/kg LE was similar (Figure 4(A)), while the sleep duration significantly increased \((p < 0.001, \text{Figure 4(B)})\) compared to that of the NC group. The group (PIX) treated with PIX, a non-competitive GABA_\text{A} receptor inhibitor, showed no significant change in sleep activity. There was no change in sleep latency even in the group administered with PIX and LE (coadministered with PIX and LE); however, the combined administration of the two significantly increased the sleep duration \((p < 0.001)\). Treatment with FLU, a chemical specifically binding to GABA_\text{A} receptor also did not increase the sleep latency, but did reduce the sleep duration. When a combination of LE and FLU was administered to mice, the sleep duration increased, implying that LE enhanced sleep-promoting activity by binding to the GABA_\text{A} receptor.

**Electroencephalogram changes by LE in insomnia model induced by caffeine**

EEG was analysed to measure the sleep-promoting effect of LE in caffeine-induced insomnia rats (Figure 5). The insomnia model was induced by caffeine, and a significant difference in wake and sleep time between the control group administered with caffeine (CC) and the NC group administered only with saline was noted \((p < 0.001, \text{Figure 5(A,B)})\). In the insomnia model, the wake time was significantly shorter, and the sleep time was significantly longer in LE-administered groups receiving 120 and 150 mg/kg dose of LE than in the caffeine control group \((p < 0.001)\). Compared with the caffeine control group, the NREM sleep time that is involved in deep sleep showed a significant increase in the LE-administered groups \((p < 0.001, \text{Figure 5(D)})\). Though LE administration showed a tendency to increase the theta and delta power in NREM sleep compared to the caffeine control group, the difference was not significant (Figure 5(E,F)).
The alleviating effect of LE in the insomnia model can thus be attributed to the increase in NREM sleep.

**Discussion**

Sleep is a physiological process in the human body and an important factor in maintaining homeostasis. It regulates the central nervous system and autonomic nervous system, which control tissue, hormonal, gene, intracellular signal transduction and movement, arousal and cognitive functions. Sleep disorders, such as lack of sleep or deprivation, act as stress factors that affect not only the physical activities but the brain functions as well (Benington 2000).

Currently, the understanding on how stress affects sleep is obscure, but it is suspected to be closely related to sleep and the...
activity of the hypothalamic-pituitary-adrenal (HPA) axis. In the early stages of sleep, slow wave sleep is dominant, and the activity of the HPA axis is the lowest and continuously suppressed. Conversely, in the second half of sleep, REM sleep dominates and the activity of HPA secretion increases, approaching a daily maximum immediately after waking up. The ventrolateral preoptic nucleus (VLPO) located in the hypothalamus acts as a switch to initiate sleep. Activated VLPO neurons secrete inhibitory neurotransmitters, such as GABA, to inhibit the areas responsible for arousal and induce sleep (Saper et al. 2005).

GabAs are activated during sleep and inhibit monoamine and histamine-secreting regions to prevent waking. The thalamic reticular nucleus also contains GABA as a neurotransmitter and generates sleep spindles (Mignot et al. 2002).

Human sleep can be divided into two types: NREM and REM, which exhibit rapid eye movement (Le Bon 2020). NREM sleep starts with N1 or N2 sleep, where the depth of sleep is relatively low, and when the delta wave of high amplitude increases, it leads to a progression to deep or N3 sleep (slow wave sleep). In general, NREM sleep is followed by REM, and the NREM-REM cycle is observed three to seven times during the normal 8 h of sleep. NREM sleep accounts for 75%–80% of the total sleep time, whereas REM sleep is associated with 20%–25% (Le Bon 2020; Patel et al. 2022). In the present study, it was noted that the oral administration of LE increases sleep time by enhancing the NREM sleep time; 150 mg/kg LE increased NREM to the extent that it accounted for 85.4% of the total sleep time (Figure 2). An increase in delta wave time indicates an increase in deep sleep time. Oral LE administration ameliorated sleep quality and promoted sleep time by increasing slow wave sleep, which corresponds to deep sleep. These findings along with the results of our previous studies that involved oral administration of extracts from lotus leaf (Kim et al. 2021) and seed (Jo et al. 2021b) to rodents show that various parts of lotus are effective in increasing sleep time. In different studies measuring the sleep activity after administration of the extracts of lotus leaf and seed, the REM sleep time was significantly reduced in the experimental groups as compared to that in the control group. NREM sleep time was increased not only with these extracts but also with the rhizome extract. On the other hand, while lotus leaf increased only the theta power, the rhizome increased not only theta but also delta power, thereby increasing the NREM sleep time. Therefore, it can be stated that LE, leaf and seed can be utilized for development of functional foods that promote sleep.

Furthermore, to evaluate the sleep-promoting activity of LE, caffeine was administered to rats to develop the insomnia model. Caffeine has been used with methylxanthine compounds to increase arousal and induce cortical activation (Bonnet and Arand 1992; Nehlig et al. 1992). Caffeine has been reported to induce arousal by blocking the action of adenosine receptors and lead to insomnia by reducing slow waves (Bonnet and Arand 1992; Panagiotou et al. 2020). In this study, the delta power in NREM corresponding to the slow wave was significantly decreased in the group treated with caffeine alone compared to the NC group (Figure 5). However, administration of 120 and 150 mg/kg LE significantly increased the NREM sleep time in the respective LE-group compared to the caffeine control group. In the caffeine-induced insomnia model, oral administration of LE increased sleep time, which can be attributed to an increase in NREM sleep time.

The sleep-promoting activity of LE was achieved by binding to the GABA_A receptor (Figure 4). Treatment with the GABA_A receptor antagonists FLU and PIX decreased the sleep-promoting activity of LE. In addition, oral LE administration increased
mRNA and protein expression of the GABA<sub>A</sub> receptor (Figure 3). Unlike FLU, a benzodiazepine site antagonist, PIX, a noncompetitive chlorine channel blocker, has been shown to reduce the sedative-hypnotic effect of alkaloids extracted from lotus leaves (Yan et al. 2015). However, unlike the lotus leaf alkaloid extract, the effect of LE was altered, as it was seen that activity of LE was reversed (sleep activity decreased) on combining with the antagonist. Although there is a marginal difference in the binding mechanism of the lotus leaf alkaloid extract and the GABA<sub>A</sub> receptor, the GABA<sub>A</sub> receptor is the binding site for LE. This difference seems to be because of the different active compounds extracted from the part used as well as the extraction method employed.

The GABA<sub>A</sub> receptor is most closely associated with sleep and consists of 19 different GABA<sub>A</sub> subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π and ρ1–3) (Everington et al. 2018). When the sleep-promoting substance binds to the GABA<sub>A</sub> receptor, the ion channel opens, resulting in an increased influx of chloride ions into the cell, causing hyperpolarisation of the cell membrane and nerve suppression, thus resulting in a sleep-sedative effect. As such, GABA<sub>A</sub> receptors play an important role in the treatment of insomnia. Numerous compounds like benzodiazepines, alcohols, barbiturates and neurosteroids, which exhibit therapeutic effects such as the inhibition and regulation of neuronal hyperactivity through GABA are known to bind to GABA<sub>A</sub> receptors (Sieghart and Sperk 2002).

It was earlier documented that the oral administration of lotus leaf alkaloids significantly increases cortical GABA concentration in a dose-dependent manner (Kim et al. 2021). Similarly, the oral administration of LE increased the GABA concentration (Figure 3). GABA is a representative inhibitory neurotransmitter in the central nervous system with a sleep-promoting function. Many antianxiety and sleeping pills, including alprazolam and diazepam, function by increasing the concentration of GABA in the brain (Griffin et al. 2013). Some sleep-promoting substances including valerenic acid, baicalin and alkaloids, are also known to increase the concentration of GABA (Shi et al. 2014).

**Conclusions**

Since several years, the LE has been used as a food source in Asian countries including Korea. Our studies showed that the oral administration of LE enhanced sleep duration by increasing NREM sleep time, and also had the ability to increase the concentration of GABA (a key neurotransmitter, in the brain) and expression of GABA<sub>A</sub> receptors. Thus, consumption of LE can help improve sleep disturbances and its extract can be developed as a functional food ingredient for patients and elderly people with sleep disturbances.

**Disclosure statement**

Hyung Joo Suh received research support from COSMAXNS INC.

**Funding**

This research was supported by COSMAXNS INC., Korea [R2018541].

**Data availability statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**References**

Bastien CH, Vallerues A, Morin CM. 2004. Precipitating factors of insomnia. Behav Sleep Med. 2(1):50–62.

Benington JH. 2000. Sleep homeostasis and the function of sleep. Sleep. 23(7):959–966.

Berkley AS. 2021. Sleep, aging, and daily functioning. Nurs Clin North Am. 56(2):287–298.

Bonnet MH, Arand DL. 1992. Caffeine use as a model of acute and chronic insomnia. Sleep 15(6):526–536.

Borras S, Martinez-Solis I, Rios JL. 2021. Medicinal plants for insomnia related to anxiety: an updated review. Planta Med. 87:738–753.

Doughnog G, Ito M. 2021. Role of ascorbic acid and p-cymene in the sleep-promoting effects of Dysphania ambrosioides essential oil via the GABAergic system in a ddY mouse inhalation model. J Nat Prod. 84(1):91–100.

Everington EA, Gibbard AG, Swinny JD, Seifi M. 2018. Molecular characterization of GABA-A receptor subunit diversity within major peripheral organs and their plasticity in response to early life psychosocial stress. Front Mol Neurosci. 11:18.

Griffin CE, Kaye AM, Bueno FR, Kaye AD. 2013. Benzodiazepine pharmacology and central nervous system-mediated effects. Ochsner J. 13(2):214–223.

Ito K, Akahoshi Y, Ito M, Kaneko S. 2016. Sedative effects of inhaled essential oil components of traditional fragrance Pogostemon cablin leaves and their structure–activity relationships. J Tradit Complement Med. 6(2):140–145.

Jo K, Kim S, Ahn Y, Suh HJ. 2021a. Effects of green lettuce leaf extract on sleep disturbance control in oxidative stress-induced invertebrate and vertebrate models. Antioxidants. 10(6):970.

Jo K, Kim S, Hong KB, Suh HJ. 2021b. Nelumbo nucifera promotes non-rapid eye movement sleep by regulating GABAergic receptors in rat model. J Ethnopharmacol. 267:113511.

Kim J, Lee SL, Kang I, Song YA, Ma J, Hong YS, Park S, Moon SI, Kim S, Jeong S, et al. 2018. Natural products from single plants as sleep aids: a systematic review. J Med Food. 21(5):433–444.

Kim S, Hong KB, Jo K, Suh HJ. 2021. Quercetin-3-O-glucuronide in the ethanol extract of lotus leaf (Nelumbo nucifera) enhances sleep quantity and quality in a rodent model via a GABAergic mechanism. Molecules 26(10):3023.

Le Bon O. 2020. Relationships between REM and NREM in the NREM-REM sleep cycle: a review on competing concepts. Sleep Med. 706:16.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>–</sup>(Delta Delta C(T)) method. Methods 25(4):402–408.

Madari S, Golebiowski R, Mansukhani MP, Kolla BP. 2021. Pharmacological management of insomnia. Neurotherapeutics 18(1):44–52.

Mignot E, Taheri S, Nishino S. 2002. Sleeping with the hypothalamus: emerging therapeutic targets for sleep disorders. Nat Neurosci. 5(S11):1071–1075.

Mukherjee PK, Saha K, Das J, Pal M, Saha BP. 1997. Studies on the anti-inflammatory activity of rhizomes of Nelumbo nucifera. Planta Med. 63(4):367–369.

Nehlig A, Daval JL, Debry G. 1992. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res Brain Res Rev. 17(2):139–170.

Ohayon MM. 2011. Epidemiological overview of sleep disorders in the general population. Sleep Med Res. 2(1):1–9.

Panagiotou M, Meijer M, Meijer JH, Deboer T. 2020. Sleep alterations in the course of aging environmental inputs [Doctoral Thesis]. Leiden: Leiden University Repository. p. 68–87.

Patel AK, Reddy V, Araujo JF. 2022. Physiology, sleep stages. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.

Saper CB, Scammell TE. 2005. Homeostatic, circadian, and emotional regulation of sleep. J Comp Neurol. 493(1):92–98.

Shi Y, Dong JW, Zhao JH, Tang LN, Zhang JF. 2014. Herbal insomnia medications that target GABAergic systems: a review of the psychopharmacological evidence. Curr Neuropharmacol. 12(3):289–302.
Sieghart W, Sperk G. 2002. Subunit composition, distribution and function of GABA(A) receptor subtypes. Curr Top Med Chem. 2(8):795–816.

Sridhar KR, Bhat R. 2007. Lotus-A potential nutraceutical source. J Agric Technol. 3:143–155.

Yan MZ, Chang Q, Zhong Y, Xiao BX, Cao FR, Pan RL, Zhang ZS, Liao YH, Liu XM. 2015. Lotus leaf alkaloid extract displays sedative-hypnotic and anxiolytic effects through GABAA receptor. J Agric Food Chem. 63(42):9277–9285.

Yang D, Wang Q, Ke L, Jiang J, Ying T. 2007. Antioxidant activities of various extracts of lotus (Nelumbo nucifera Gaertn) rhizome. Asia Pac J Clin Nutr. 16:158–163.

Zapata-Sudo G, Mendes TCF, Kartnaller MA, Fortes TO, Freitas NFB, Kaplan MAC, Sudo RT. 2010. Sedative and anticonvulsant activities of methanol extract of Dorstenia arifolia in mice. J Ethnopharmacol. 130(1):9–12.

Zhao X, Shen J, Chang KI, Kim SH. 2014. Comparative analysis of antioxidant activity and functional components of the ethanol extract of lotus (Nelumbo nucifera) from various growing regions. J Agric Food Chem. 62(26):6227–6235.