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Effects of *Cynanchum Wilfordii* Hemsley Extract on the Sleep-Wake Architectures in Rats

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Background and Objective Although *Cynanchum wilfordii* Hemsley *(C. wilfordii)* has been widely used for treating insomnia in Korea, there are no objective data on its sleep-enhancing effects.

Methods We examined the effects of this extract on sleep latency, sleep parameters, and NREM delta activity in rats administered several different dosages (300 mg/kg, 1 g/kg or 3 g/kg, p.o.) and compared them to valerian extract which is a popular natural hypnotic.

Results We found no significant shortening of sleep latency was observed with any dosage of either *C. wilfordii* extract or with valerian. NREM sleep increased significantly at *C. wilfordii* dosages of 300 mg/kg and 1 g/kg but not for 3 g/kg. Only the 1 g/kg dosage significantly decreased the amount of wakefulness. Neither any of the *C. wilfordii* dosages nor valerian extract produced any effect on REM sleep amount.

Conclusions Therefore, *C. wilfordii* could be a useful natural hypnotic with a sleep-enhancing effect.

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Key Words *Cynanchum wilfordii* Hemsley, Valerian, Insomnia, Sleep-enhancing effect, Sleep latency, NREM delta activity.

INTRODUCTION

Insomnia is a very common health problem affecting approximately 10-30% of the population in Western countries and approximately 23% of Korean adults.[1-4] Medications based on chemical hypnotics, such as benzodiazepines, have the following adverse effects: diurnal 'hangover' effects, dependence, addiction, withdrawal symptoms, and subsequent drug-resistance.[5] The demand for complementary and alternative medicines for treating insomnia is increasing, not only because patients wish to avoid the aforementioned adverse effects, but also because herbal/natural products are readily available and require no prescription from a doctor, in contrast to chemical drugs.[6-8]

Cynanchum wilfordii Hemsley *(C. wilfordii)*(Asclepiadaceae) is a perennial herb, whose roots have been widely used in Korea for treating insomnia, anxiety, anemia, senescence, and various geriatric diseases.[9,10] However, despite its wide use in Korea, no objective studies evaluating its efficacy in treating or preventing insomnia exist. Therefore, in the present study, we evaluated *C. wilfordii* extract's effects on the sleep-wake cycle in rats and compared it to valerian extract, an herbal product in widespread use for treating insomnia, to evaluate its possibilities as a natural hypnotic.

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METHODS

Animals

Thirty five Male Sprague-Dawley rats (Orient Bio Inc, Seong-Nam, Korea) weighing 230-310 g were used in this study. All animals were maintained in a temperature-controlled recording room with a 12/12 light/dark cycle (lights on at 7 : 00 a.m.) and ad libitum food and water. Room temperature (24 ± 2°C) and humidity (50 ± 20%) were monitored continuously. All experimental procedures involving animals were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Committee of the Korea University College of Medicine.

Surgery

The animals were implanted with chronic recording devices for continuously recording electroencephalography (EEG) and electromyography (EMG). With the animal under isoflurane anesthesia (induction 5%, maintenance 2-2.5%), holes were drilled through the skull bilaterally at -5.0 mm AP and 2.0 mm ML from the bregma. For the EEG, two stainless steel screws were inserted into the holes, and a screw fixed in the left frontal bone served as a reference. For the EMG, implanted stainless steel wire electrodes were sutured into the dorsal neck muscle. EEG and EMG electrodes were then connected to a miniature connector and affixed to the skull with dental cement. After surgery, the animals were placed in clean cages and allowed at least 14 days to recover from surgery.

EEG and EMG Recordings

After recovery from surgery, the animals were connected to a cable and acclimatized to the handling procedures, and were given a mock dosing for 3 days before the experimental day. EEG and EMG signals were recorded and amplified on a digital polygraph (PowerLab, ADInstruments, NSW2153, Australia) at a sampling rate of 200 Hz. EEG and EMG filter ranges were set at 0.3-30 Hz and 10-200 Hz, respectively. After data collection ended, sleep-wake states were semi-automatically scored into 10-second epochs as waking, NREM sleep, or REM sleep by examination of EEG and EMG recordings, and were then confirmed by visual scoring using SleepSign software (Kissei Comtec, Nagano, Japan). Each vigilance state was determined after taking into account visual and power spectrum value as follows: wake, low-amplitude EEG, and high-voltage EMG activities; NREM sleep, high-amplitude slow or spindle EEG, and low-EMG activities; and REM sleep, low-voltage EEG, and EMG activities.

Experimental Design

Dosing conditions comprised 3 differing dosages of *C. wilfordii* extract (300 mg/kg, 1 g/kg and 3 g/kg; provided by the Korea Food Research Institute), 1 valerian extract dosage (1 g/kg), and a vehicle control (0.9% physiologic saline), all administered in a counterbalanced, random design. Each group contained 5 rats.

The *C. wilfordii* extract was prepared in 78% ethanol. Other sleep studies on the rat have employed the valerian dosage we used. Dosing solutions were prepared in 0.9% physiologic saline and administered orally at 9 : 00 a.m., via gavage needle. After administration, we allowed the rats 15 min to stabilize and then recorded their EEGs and EMGs for 6 h.

Data and Statistical Analysis

We analyzed the EEG and EMG data, scoring it into 10-second epochs as waking, NREM sleep, or REM sleep, and expressed the data as time spent in each state. Sleep latency was defined as the time from drug administration until the first 12 continuous 10-second epochs were scored as sleep. A "bout" consisted of a minimum of 2 consecutive 10-second epochs of a given state and ended with any single state-change epoch. To analyze the EEG spectra during NREM sleep, we used a fast Fourier transformation algorithm (SleepSign software, Kissei Comtec, Nagano, Japan) on all epochs without visually detectable artifact. The EEG delta power (0.5-4 Hz) within NREM sleep was analyzed in hourly bins and expressed as a percentage of the average delta activity in NREM sleep during the entire recording period for each group.

Values shown are means ± SEM. Differences between treated and control groups in all tests were analyzed using a Kruskal-Wallis analysis of variance on ranks, followed by the Mann-Whitney's U test. All statistical analyses were carried out using SPSS, version 13.0 (SPSS, Inc., Chicago, IL, USA). A p value of < 0.05 was considered statistically significant.

RESULTS

Sleep Latency

Relative to the vehicle controls, no *C. wilfordii* extract dosages showed a significant effect on sleep latency (Fig. 1). Valerian extract also had no significant effect on sleep latency.

Wakefulness, NREM Sleep, and REM Sleep Amounts

Cumulative NREM sleep increased significantly, compared to controls, with *C. wilfordii* extract treatment at dosages of 300 mg/kg and 1 g/kg, but not at 3 g/kg. Time spent in the waking stage decreased significantly, relative to the NREM sleep increase, at doses of both 300 mg/kg and 1 g/kg. Valerian also increased NREM sleep compared to controls, but its effect on NREM sleep did not differ significantly from that of *C. wilfordii* extract. We found no significant difference was found in the amount of NREM sleep between any *C. wilfordii* dosage and valerian extract. Neither *C. wilfordii*, at any dosage, nor valerian extract affected the amount of REM sleep. The highest *C. wilfordii* ex-

tract dosage (3 g/kg) produced less REM sleep time compared to controls or valerian treatment; however, this difference was not significant (Table 1).

NREM Delta Activity

Table 2 shows the delta activity during NREM sleep. None of the *C. wilfordii* extract dosages produced any significant change in NREM delta power over the entire period after treatment. Valerian also had no significant effect on NREM delta activity (Table 2).

Number and Mean Duration of Episodes

Additionally, 300 mg/kg and 1 g/kg dosages of *C. wilfordii* and also valerian extract significantly affected measures of sleep-wake consolidation. Although waking bout number did not differ statistically, the waking bout duration decreased at *C. wilfordii* dosages of 300 mg/kg and 1 g/kg (Table 3). On the other hand, both 300 mg/kg and 1 g/kg failed to prolong NREM sleep duration, yet they increased NREM sleep bouts as the waking bout duration decreased (Table 4). Neither any dosages of *C. wilfordii* nor valerian affected either REM sleep bout duration or the number of REM bouts (Table 5).

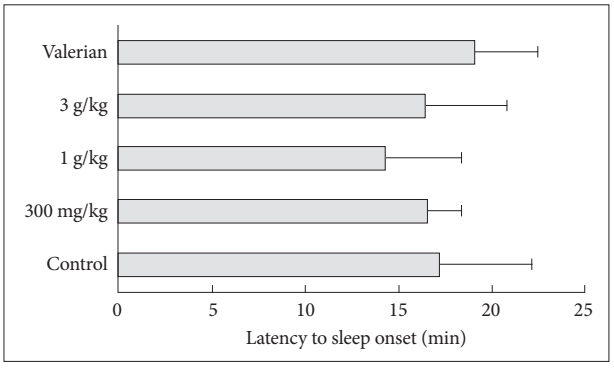

Fig. 1. Effects of *Cynanchum wilfordii* Hemsley extract, valerian, and vehicle on sleep latency in rats. There was no significant difference in sleep latency of rats treated with all doses of *C.wilfordii* Hemsley, valerian and vehicle. Mean (± SEM) sleep latency was defined as the first 12 continuous epochs of sleep following drug administration. n = 5 in each group.

DISCUSSION

Despite widespread use of *C. wilfordii* as a natural hypnotic in traditional Korean medicine, no objective reports on its efficacy in treating insomnia are available. Thus, the present study investigated the sleep-enhancing effects of *C. wilfordii* extract in rats. Results showed that *C. wilfordii* extract increased NREM sleep time, without any significant change in NREM delta activity. Although dosages of 300 mg/kg and 1 g/kg significantly increased NREM sleep time, we did not observe this effect with the markedly higher dosage of 3 g/kg, suggesting that an optimal *C. wilfordii* extract concentration for increasing sleep exists. *C. wilfordii* seems to affect sleep states and NREM delta activity in different ways, in that no dosages of *C. wilfordii* extract changed the latter. We also evaluated *C. wilfordii* in regard to sleep latency in rats, but we did not find any significant effect. This study compared sleep parameters after valerian extract treatment to the same parameters after several different *C. wilfordii*

Table 1. Cumulative amount of waking, NREM, and REM sleep for 6 hours following treatment with *Cynanchum wilfordii* Hemsley extract, valerian, or vehicle

State	Vehicle	*Cynanchum wilfordii* Hemsley extract			Valerian
		300 mg/kg	1 g/kg	3 g/kg	1 g/kg
Waking	132.5 ± 9.7	103.5 ± 7.6*	94.5 ± 12.7*	119.1 ± 9.0	102.0 ± 12.7*
NREM	194.8 ± 7.8	226.7 ± 5.7*	232.5 ± 9.0*	214.9 ± 6.2	225.8 ± 8.8*
REM	32.6 ± 8.5	29.6 ± 4.3	32.8 ± 4.2	25.8 ± 3.9	32.0 ± 4.4

Data are presented as means ± SEM (n = 5), in minutes.
*Significantly different from vehicle at $p < 0.05$.
NREM: non-rapid eye movement sleep, REM: rapid eye movement sleep.

Table 2. Effects of *Cynanchum wilfordii* Hemsley extract, valerian, or vehicle on delta activity during NREM sleep in rats

Dose (p.o)	EEG activity (% of control)					
	0-1	1-2	2-3	3-4	4-5	5-6 (h)
Cynanchum wilfordii Hemsley extract						
Vehicle	100 ± 15.7	100 ± 12.8	100 ± 7.9	100 ± 14	100 ± 10.1	100 ± 7.2
300 mg/kg	92.9 ± 42.1	92.9 ± 43.9	105.2 ± 45.5	88.3 ± 39.5	81.3 ± 33.5	98.9 ± 37.8
1 g/kg	65.2 ± 22.3	74.0 ± 24.2	84.7 ± 26.2	90.3 ± 27.0	82.5 ± 25.5	85.0 ± 23.0
3 g/kg	113.8 ± 33.6	139.9 ± 40.3	176.6 ± 37.2	151.3 ± 29.3	165.4 ± 45.8	180.4 ± 37.0
Valerian	87.5 ± 19.7	102.2 ± 34.0	119.4 ± 35.1	103.3 ± 27.7	95.8 ± 23.8	99.0 ± 18.5

Data are presented as means ± SEM (n = 5).
NREM: non-rapid eye movement sleep, EEG: electroencephalography.

Table 3. Duration of wake time and number of wake bouts observed per hour following treatment with *Cynanchum wilfordii* Hemsley extract, valerian, or vehicle

Time (h)	Vehicle	*C. wilfordii* Hemsley			Valerian
		300 mg/kg	1 g/kg	3 g/kg	1 g/kg
Wake bout duration (sec)					
1	68.3 ± 26.0	55.5 ± 6.1†	45.6 ± 4.9	42.0 ± 5.9	41.8 ± 4.3
2	29.8 ± 3.5	37.0 ± 7.2	39.4 ± 7.2	32.6 ± 9.5	25.4 ± 1.5
3	92.5 ± 33.7	39.3 ± 3.6	48.8 ± 10.1	61.2 ± 16.8	26.8 ± 4.2*
4	27.3 ± 2.5	34.5 ± 8.5	32.2 ± 6.1	48.0 ± 11.8	36.4 ± 8.1
5	104.3 ± 63.0	42.0 ± 10.4	27.0 ± 5.4	44.8 ± 12.4	38.6 ± 11.0
6	56.5 ± 19.1	31.3 ± 4.6	43.2 ± 4.5†	31.4 ± 4.5	24.4 ± 5.0*
Number of bouts					
1	26.3 ± 3.7	33.0 ± 5.0	29.0 ± 2.6	32.6 ± 3.7	30.4 ± 1.7
2	28.8 ± 1.6	27.8 ± 2.7	21.8 ± 3.1	26.2 ± 4.1	24.4 ± 5.6
3	28.0 ± 10.2	29.0 ± 2.1	24.0 ± 2.7	23.4 ± 4.3	24.0 ± 3.3
4	27.8 ± 1.9	31.0 ± 3.3	23.8 ± 2.2	27.2 ± 4.9	26.4 ± 4.4
5	22.0 ± 4.8	31.5 ± 3.7	23.2 ± 1.1	25.4 ± 2.7	26.0 ± 3.5
6	30.0 ± 4.3	24.5 ± 1.0	23.6 ± 2.0	26.4 ± 2.8	22.8 ± 2.9

Data are presented as means ± SEM (n = 5).
*Significantly different from vehicle at $p < 0.05$, †Significantly different from valerian at $p < 0.05$.

Table 4. Duration of NREM sleep time and number of NREM bouts observed per hour following treatment with *Cynanchum wilfordii* Hemsley extract, valerian, or vehicle

Time (h)	Vehicle	*C. wilfordii* Hemsley			Valerian
		300 mg/kg	1 g/kg	3 g/kg	1 g/kg
NREM bout duration (sec)					
1	84.0 ± 9.5	91.5 ± 9.8	79.6 ± 17.2	92.4 ± 12.1	83.6 ± 5.9
2	93.8 ± 13.5	99.0 ± 10.6	97.6 ± 22.1	129.0 ± 18.0	134.0 ± 13.21
3	101.0 ± 18.1	95.8 ± 18.0	85.2 ± 8.3	130.0 ± 16.3	123.2 ± 14.1
4	88.8 ± 6.7	91.8 ± 15.6	112.4 ± 15.1	100.4 ± 7.6	110.8 ± 10.1
5	104.5 ± 10.8	89.8 ± 8.2	101.2 ± 7.9	112.4 ± 9.9	94.4 ± 9.4
6	110.0 ± 28.6	110.5 ± 7.7	101.6 ± 17.1	105.0 ± 12.1	118.8 ± 10.7
Number of bouts					
1	23.3 ± 4.5	27.5 ± 5.7	25.0 ± 4.3	24.8 ± 1.6	26.0 ± 2.0
2	26.0 ± 3.3	25.5 ± 4.2	23.2 ± 3.4	20.2 ± 1.6	19.6 ± 1.7
3	15.8 ± 3.3	25.5 ± 3.6	21.8 ± 1.4	17.2 ± 1.2	20.8 ± 1.7
4	27.0 ± 2.0	26.3 ± 4.7	23.6 ± 2.2	20.4 ± 1.3*	19.8 ± 2.9
5	17.0 ± 2.9	24.8 ± 3.1	23.4 ± 1.0*	20.0 ± 1.8	23.0 ± 1.4
6	18.5 ± 3.4	21.0 ± 2.0	21.8 ± 2.2	22.2 ± 0.6	20.6 ± 1.8

Data are presented as means ± SEM (n = 5).
*Significantly different from vehicle at $p < 0.05$.
NREM: non-rapid eye movement sleep.

dosages. However, we did not observe a significant shortening of sleep latency like that in the reports from Tokunaga et al., in which 1 g/kg of valerian resulted in a shorter sleep latency.[11]

To the best of our knowledge, no reports exist yet on the effect of *C. wilfordii* on the central nervous system in rats yet or on its sleep-enhancing effect. Only a few studies have reported its neuroprotective effect against oxidative stress in cultured cortical neurons[12] and hepatocytes.[13] Although we found in our *in vitro* competitive receptor binding assay (data not shown) that homogenates of several rat brain regions, related to sleep regulation, have a high affinity for 5-HT_{2c} ligands, the exact mechanisms whereby *C. wilfordii* has its sleep-enhancing effect are not clear yet. Further studies are required to clarify them.

No toxicity test was performed in this study, but this is ne-

Table 5. Duration of REM sleep time and number of REM bouts observed per hour following treatment with *Cynanchum wilfordii* Hemsley extract, valerian, or vehicle

Time (h)	Vehicle	*C. wilfordii* Hemsley			Valerian
		300 mg/kg	1 g/kg	3 g/kg	1 g/kg
REM bout duration (sec)					
1	77.5 ± 31.2	40.0 ± 26.1	104.0 ± 38.1	110.6 ± 17.0	117.6 ± 13.9
2	116.3 ± 13.4	100.5 ± 17.4	127.6 ± 14.5	150.2 ± 25.9	119.2 ± 13.6
3	121.5 ± 33.7	100.3 ± 16.6	113.0 ± 16.0	98.6 ± 24.1	102.2 ± 6.0
4	81.0 ± 7.1	101.0 ± 12.1	123.2 ± 12.1*	74.6 ± 26.8	128.6 ± 22.3*
5	85.8 ± 10.6	84.8 ± 18.2	136.0 ± 15.3*	95.0 ± 29.0	127.8 ± 10.0*
6	86.8 ± 14.0	113.5 ± 10.4	88.8 ± 14.8	70.8 ± 7.4†	114.4 ± 9.1
Number of bouts					
1	2.3 ± 1.0	0.8 ± 0.8	1.6 ± 0.5	2.2 ± 0.8	1.6 ± 0.4
2	3.8 ± 0.9	3.0 ± 0.8	3.4 ± 0.9	3.2 ± 0.6	3.4 ± 0.8
3	2.0 ± 0.4	2.8 ± 0.6	2.4 ± 0.5	2.0 ± 0.8†	4.6 ± 0.7*
4	6.0 ± 2.0	3.0 ± 1.4	2.4 ± 0.5*	2.0 ± 0.6*	4.2 ± 0.9
5	3.8 ± 1.8	3.0 ± 1.1	3.8 ± 0.9	2.6 ± 1.2	3.8 ± 0.7
6	3.3 ± 1.4	5.5 ± 0.5	4.0 ± 0.6	3.4 ± 0.9	5.0 ± 0.5

Data are presented as means ± SEM (n = 5).
*Significantly different from vehicle at $p < 0.05$, †Significantly different from valerian at $p < 0.05$.
REM: rapid eye movement sleep.

cessary for any consideration of its pharmaceutical application in humans, especially since dosages used in this study were rather high.

In conclusion, our present results indicate that *C. wilfordii* extract has significant sleep-enhancing effects and may prove useful as a natural hypnotic for treating insomnia by increasing NREM sleep duration. However, the small number of animals tested could be a limitation of this study. Further studies are needed to clarify the exact *in vivo* mechanism and safety of *C. wilfordii*.

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Conflicts of Interest

The authors have no financial conflicts of interest.

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