Biomedicine & Pharmacotherapy 105 (2018) 167–175

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Polygonatum sibiricum rhizome promotes sleep by regulating non-rapid eye movement and GABAergic/serotonergic receptors in rodent models

Kyungae Jo[a], Hyung Joo Suh[a], Hyeon-Son Choi[b,*]

[a] Department of Public Health Science, Korea University, Seoul 02841, Republic of Korea
[b] Department of Food Science and Technology, Seoul Women's University, Seoul 01797, Republic of Korea

ARTICLE INFO

Keywords:
Polygonatum sibiricum
Insomnia
Sleep
Caffeine
γ-aminobutyric acid
Vertebrate

ABSTRACT

The aim of this study is to investigate the sleep-promoting effect of a water extract of the *Polygonatum sibiricum* rhizome (PSE) in rodent models. PSE contained oleamide (0.10 mg/g extract) and glyceryl monolinoleate (0.17 mg/g extract), which are recognized as sleep-promoting substances. In pentobarbital-induced sleep model at hypnotic level, PSE (160 mg/kg) administration significantly decreased sleep latency time by 29% (2.7 min) and increased sleep duration time by 70% (68.4 min) compared with the normal control (3.8 min and 40.7 min, respectively). In the electroencephalography (EEG) analysis of rats, PSE-mediated sleep promotion accompanied the change of sleep architecture including increase of non-rapid eye movement (NREM) and decrease of REM. This sleep promoting effect was more obvious in caffeine-induced awakening model; total sleep time was increased by 40% along with increased NREM by PSE treatment at 160 mg/kg. In addition, PSE significantly increased the protein and mRNA levels of $GABA_A$-R2 and 5-HT1A receptor, the major sleep-related neurotransmitter receptors. Furthermore, glyceryl monolinoleate and oleamide effectively bound to $GABA_A$ receptor in a competitive binding assay. These results indicate that PSE-mediated sleep-promoting effect is associated with the extension of NREM and upregulation of $GABA_A$-R2 and 5-HT1A, and is mediated by binding to the $GABA_A$ receptor in vertebrate models.

1. Introduction

Polygonatum sibiricum, also known as Solomon's seal, is a flowering plant belonging to the family Liliaceae [1]. It is native to East Asian countries including China and Korea [1]. Most parts of the plant are used for food in raw and cooked forms [2]. In particular, the rhizome of *P. sibiricum* called Huang Jing has been applied to prepare tea or as a flavoring agent for the beverage [2]. The tea made from the rhizome is called *dungulle* in Korea. This *Polygonatum* rhizome has been commonly used in traditional Chinese medicine in herbal remedies [1]. The use of *P. sibiricum* in folk remedies for a long time has proven its effectiveness and safety [2]. A recent study showed that repeated administration of 80% ethanol extract of the dried *P. sibiricum* rhizome to Sprague-Dawley rats resulted in the No-Observed-Adverse-Effect-Level in even more than 2000 mg/kg dosage, indicating no toxicity of *P. sibiricum* [3]. Many studies have shown physiological activities from *Polygonatum* rhizome; hypoglycemic [4] and cardiotonic [5] activities, a reduction in blood glucose and lipid levels, enhancement of the immune system, and antiaging [6] and antioxidant/anti-inflammatory [1] activities. However, the sleep-promoting effect of *P. sibiricum* rhizome is yet to be studied.

Sleep plays an important role in maintaining a healthy lifestyle [7]. The quantity and quality of sleep are closely related to mental/physical health and safe life [7]. Sleep helps the brain activity properly in learning and remembering information [7]. It also supports normal growth and development by maintaining a hormonal balance [8]. Insomnia is one of the common sleep disorders where people have difficulty falling asleep or maintaining sleep [9]. Insomnia has been an increasing health issue worldwide. Approximately 30% of the general population is known to experience insomnia symptoms during their lifetime [10]. Long-lasting sleep deficiency has been linked to various health problems such as heart disease, high blood pressure, stroke, and obesity [9]. In addition, insomnia is involved in occupational or automobile accidents [9]. Insomnia treatment can be classified into two main categories: pharmacological treatments and nonpharmacological treatments. For pharmacological treatment, benzodiazepine (BDZ) drugs are the most widely used sedative hypnotics, which induce sleep [11]. Nonetheless, the continuous use of such a drug can have adverse effects including deterioration of daytime performance, memory loss, drug resistance, and addiction [12]. Recently, nonpharmacological

* Correspondence author.
E-mail address: hschoi@swu.ac.kr (H.-S. Choi).

https://doi.org/10.1016/j.biopha.2018.05.115
Received 28 February 2018; Received in revised form 21 May 2018; Accepted 23 May 2018

treatments involving plants (so-called natural remedies) have been studied and suggested as a new approach with milder side effects. These herbal remedies have also been shown to regulate central GABAergic neurotransmission to promote sleep [13].

Phytotherapy has been traditionally used to treat insomnia in various countries worldwide for thousands of years [13]. Many studies have shown that natural products may be useful for the management of sleep disorders, e.g., various models of administration of valerian (*Valeriana officinalis*) roots are known to reduce sleep latency and wake time after sleep onset in humans [14]. A hydro-alcoholic extract of lettuce (*Lactuca sativa*) has been shown to prolong pentobarbital-induced sleep duration in mice [15]. The present study was designed to investigate the effect of *P. sibiricum* rhizome water extract (PSE) on sleep and related behaviors in rodent models. For the analysis of sleep quality, the effect of PSE on brain waves was studied by electroencephalography (EEG). In addition, $GABA_A$ receptor binding activity of PSE and possible sleep-promoting substances were evaluated. The present study may be the first to show a sleep-promoting effect of the *P. sibiricum* rhizome.

2. Materials and methods

2.1. Plant material and preparation of extracts

A *P. sibiricum* rhizome (100 g) was extracted with 700 mL of distilled water at 80℃ for 2 h. This process was repeated for 1 h with fresh distilled water, and the combined aqueous extracts were then filtered, concentrated by vaporization under reduced pressure at 60℃ in a rotary evaporator (R-100, BUCHI Labortechnik AG, Flawil, Switzerland), freeze-dried, and stored at 4 ℃.

2.2. Analysis of polyphenol/flavonoids, sugars, and oleamide/glyceryl monolinoleate

Total polyphenol content was determined by the Folin-Ciocalteu method [16]. The absorbance at 750 nm was read, and the readings were interpolated using a gallic acid calibration curve to determine the total polyphenol content, which was expressed as μg/mL of gallic acid.

Total flavonoid content was calculated using the *p*-dimethylaminocinnamaldehyde (DMACA) method [17]. The readings were interpolated using a catechin calibration curve to determine the total flavonoid content, which was expressed as μg/mL of catechin.

Total sugar contents were determined by method [18] with some modifications. Glucose was used as a standard material for external standard method.

For analysis of oleamide and glyceryl monolinoleate, 100 mg of PSE was subjected to acid hydrolysis and saponification. The PSE sample was treated with hydrochloric acid (2 N) in a shaking water bath at 75 ℃ and 80 rpm for 100 min. After that, it was cooled at room temperature and purged with nitrogen gas. The acid-hydrolyzed sample was mixed with 4 N methanolic KOH and was saponified under the same reaction conditions, followed by addition of 50% methanolic KOH for complete saponification [19]. For extraction of oleamide and glyceryl monolinoleate, distilled water with chloroform (1:1) was added, and the mixture was shaken vigorously. After centrifugation at 2500 rpm for 3 min (1248R, Labogene, Denmark), the water layer was removed. This clean-up procedure was repeated four times, and the lower layer was chosen as the analytical sample.

Sialylation of the sample was performed with 100 μL of *N,O*-bis (trimethylsilyl)trifluoroacetamide (BSTFA) and 1% trimethylchlorosilane (TMCS) to analyze glyceryl monolinoleate by GC. After a reaction at 60 ℃ and 80 rpm for 1 h in a shaking water bath, the mixture was cooled at room temperature, purged with nitrogen gas, dissolved in chloroform, and injected into a gas chromatograph. The chromatographic separation was performed on a GC-14B instrument (Shimadzu, Tokyo, Japan) equipped with a flame ionization detector and a SAC-5 column (0.25 μm film thickness × 0.25 mm diameter × 30 m length). Helium served as a carrier gas at a flow rate of 12.6 mL/min. The oven temperature was maintained at 250 ℃ for 1 min and increased to 320℃ at 10 ℃/min (holding time, 8 min). The samples (1 μL) were injected at a split ratio of 10:1 and an injector temperature of 310 ℃.

The oleamide analysis was carried out on an HPLC system (Waters Scientific Ltd., Mississauga, Canada) equipped with an Eclipe Plu C18 column (4.6 mm × 100 mm, Agilent, Santa Clara, CA 95051, USA) at a wavelength of 205 nm. The mobile phase consisted of the mixture acetonitrile:distilled water (8:2) at a flow rate of 1.0 mL/min with an injection volume of 10 μL [20].

2.3. Animals

Male ICR mice (4 weeks old, 18–22 g) and Sprague–Dawley rats (8 weeks old, 180–200 g) were acquired from Orient Bio (Orient Bio Inc., Seongnam, Korea). All the animals were housed in cages at 24 ℃ and 55% relative humidity in a 12 h light/dark cycle. Food and water were freely available. The rodents were acclimated to the vivarium for at least 1 week before a pentobarbital-induced sleep test and electroencephalographic analysis were started. All the animal experiments were approved by the Korea University Animal Care Committee (KUIACUC-2017-49, Seoul, Korea).

2.4. The pentobarbital-induced sleep test

All these experiments were carried out between 1:00 and 5:00 pm, and mice were fasted for 24 h prior to the experiment. For oral administration, all samples were resuspended in physiological saline. The groups were administered benzodiazepine (BDZ, 2.5 mg/kg), PSE (80 and 160 mg/kg), and then, 45 min later, pentobarbital (a subhypnotic dose, 35 mg/kg, and a hypnotic dose, 42 mg/kg) was injected into the left side of the abdomen. After injection, the mice were placed in individual cages and subjected to measurements of sleep latency and duration. Sleep latency was defined as the period between pentobarbital injection and sleep onset, and sleep duration denotes the time elapsed between the righting reflex loss and recovery [21]. Mice that failed to fall asleep within 10 min after pentobarbital injection were excluded from the experiments.

2.5. EEG recordings and analysis

Rats were anesthetized with 2% isoflurane (Troikaa Pharmaceutical Ltd., Gujarat, India), then maintained with 1% isoflurane in an oxygen/air mixture using a gas anesthesia mask in a stereotaxic instrument frame. The top of a rat's head overlying the surgical area was shaven, cleaned, and disinfected with 70% ethanol. The incision in midline skin was made, exposing the skull. Four holes were drilled in the skull, and small screws were inserted into the skull, which corresponded to the striatum, cortex, and hippocampus. The electrodes and socket were fixed with dental cement. All the rats were medicated with an antibiotic and individually housed in cages at a temperature-controlled facility with water and food. After 7 days for recovery, the rats were randomly subdivided into control and treatment groups. The experiments were conducted in the daytime between 10 a.m. and 5 pm for 9 days. Samples were administered orally 1 h before the experimental analysis; EEG signals were amplified, filtered (0.5–30.0 Hz), recorded, and stored using Iox2 (version 2.8.0.13, emka Technologies, Paris, France). EEG spectra were analyzed in 1 Hz frequency bins and in standard frequency bands (γ: 30–60 Hz; β: 12–30 Hz; α: 9–12 Hz; theta (θ): 4–9 Hz; δ: 0.5–4 Hz). After each recording, fast Fourier transform (FFT) was performed every 2 s, and the FFT data were averaged in the range of 0–30 Hz for 10 s intervals to calculate the wake and sleep time in the ecgAUTO3 software (version 3.3.0.20, emka Technologies). Caffeine (10 mg/kg) was employed to induce wakefulness before the experiments.