

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Sedative and hypnotic effects of supercritical carbon dioxide fluid extraction from *Schisandra chinensis* in mice

Hongyan Zhu, Lina Zhang, Guoli Wang, Zhongmei He, Yan Zhao, Yonghua Xu, Yugang Gao, Lianxue Zhang*

College of Chinese Medicinal Materials, Jilin Agricultural University, Changchun, China

ARTICLE INFO

Article history:

Received 24 November 2015

Received in revised form

22 April 2016

Accepted 13 May 2016

Available online 15 July 2016

Keywords:

GABAergic system

hypnotic effect

sedative effect

Schisandra chinensis

serotonergic system

supercritical carbon dioxide

ABSTRACT

Schisandra chinensis is a traditional Chinese medicine that has been used for treating insomnia and neurasthenia for centuries. Lignans, which are considered to be the bioactive components, are apt to be extracted by supercritical carbon dioxide. This study was conducted to investigate the sedative and hypnotic activities of the supercritical carbon dioxide fluid extraction of *S. chinensis* (SFES) in mice and the possible mechanisms. SFES exhibited an obvious sedative effect on shortening the locomotor activity in mice in a dose-dependent (10–200 mg/kg) manner. SFES (50 mg/kg, 100 mg/kg, and 200 mg/kg, intragastrically) showed a strong hypnotic effect in synergy with pentobarbital in mouse sleep, and reversal of insomnia induced by caffeine, *p*-chlorophenylalanine and flumazenil by decreasing sleep latency, sleep recovery, and increasing sleeping time. In addition, it produced a synergistic effect with 5-hydroxytryptophan (2.5 mg/kg, intraperitoneally). The behavioral pharmacological results suggest that SFES has significant sedative and hypnotic activities, and the mechanisms might be relevant to the serotonergic and γ -aminobutyric acid (GABA)ergic system.

Copyright © 2016, Food and Drug Administration, Taiwan. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Schisandra chinensis, recorded as the ripe fruits of *Schisandra chinensis* (Turcz.) Baill. in the Chinese Pharmacopoeia, has been used as a common traditional Chinese medicine for treating asthmatic coughing, spontaneous or night sweating, body fluid deficiency, palpitation, and insomnia [1]. There are

many reports on its chemical constituents, which are mainly lignans, polysaccharides, and organic acids [2,3]. The dibenzocyclooctene lignans, such as schisandrol A, deoxyschizandrin, schisantherin A, schisandrin B, and schisandrin C, are the major bioactive constituents for the hepatoprotective, anticancer, antiasthmatic, antiinflammatory, sedative, hypnotic and anxiolytic effects, as well as the preventive effect on Alzheimer's disease [4–16]. The ripe fruits of *Schisandra*

* Corresponding author. College of Chinese Medicinal Materials, Jilin Agricultural University, Changchun 130118, China.

E-mail address: zlxbooksea@163.com (L. Zhang).

<http://dx.doi.org/10.1016/j.jfda.2016.05.005>

1021-9498/Copyright © 2016, Food and Drug Administration, Taiwan. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

sphenanthera Rehd. et Wils. are also recorded in the Chinese Pharmacopoeia, whose composition and content of lignans are different from *S. chinensis* because of their different localities [17]. The sedative and hypnotic effects of the ethanol fraction of *S. sphenanthera* Rehd. et Wils. has been confirmed through the open field test and the potential pentobarbital-induced sleep test [18]. In fact, *S. chinensis* was more often used for clinical treatment of insomnia and neurasthenia rather than *S. sphenanthera* in China. Therefore, this study aimed to determine the sedative and hypnotic effects of *S. chinensis*, and to explore its possible mechanisms.

Supercritical carbon dioxide extraction is widely applied in pharmaceutical plants for its advantages in absence of solvent, and high extraction efficiency [19,20]. Lignans of *S. chinensis* have been extracted efficiently from the seeds, fruits, caulomas, and leaves by supercritical carbon dioxide [21,22]. *Schisandra* lignans were extracted by supercritical carbon dioxide, and their hypnotic effects were superior to ethanol extraction in subsequent experiments.

2. Materials and Methods

2.1. Animals

Male Kunming mice (22–24 g) and Wister rats (200–220 g) were purchased from Experimental Animal Center of Jilin University, Changchun, China, and housed in a temperature-controlled ($25 \pm 1^\circ\text{C}$) animal room under a 12-hour light/dark cycle with free access to water and food. The animals were acclimatized to the environment for 1 week, and then they were divided into different groups randomly with 10 mice/rats each. All experiments were carried out between 09:00 and 11:00 hours in a soundproof room, and in compliance with the National Institutes of Health and institutional guidelines approved by the Animal Care Committee of Jilin Agricultural University.

2.2. Materials and extraction

S. chinensis was collected from Jilin Province, China and identified by Professor Lianxue Zhang (College of Traditional Chinese Medicinal Materials, Jilin Agricultural University). Dried and pulverized fruits of *S. chinensis* (1 kg) were extracted by supercritical carbon dioxide fluid, with 30 Mpa, 45°C and 60 kg CO_2/h for 3 hours. Finally, the yellow oil-like extract was collected as superfluid extract of *S. chinensis* (SFES), and the yield of SFES was 4.16% (w/w). Dried and pulverized fruits of *S. chinensis* (100 g) were extracted with 95% ethanol by refluxing for 2 hours, and the ethanol extract was obtained after concentration in vacuum as the ethanol extract of *Schisandra chinensis* (SEES), and the yield of SEES was 22.1% (w/w).

2.3. Drugs and treatments

Schisandrol A, schisandrol B, schisantherin A, schisandrin A, schisandrin B, and schisandrin C were purchased from Yuan-ye Biotech Co. Ltd. (Shanghai, China). Acetonitrile (HPLC grade) was purchased from Honeywell Burdick & Jackson (Ulsan, Korea). Ultrapure water was prepared in a Milli-Q Water Purification System (Millipore, Bedford, MA, USA).

Sodium pentobarbital was purchased from Dingguo Changsheng Biotech Co. Ltd. (Beijing, China), with 42 mg/kg (intraperitoneally; i.p.) as the hypnotic dose in the experiments that could generate 100% rate of sleep onset, and 28 mg/kg (i.p.) as the subhypnotic dose. Caffeine (7.5 mg/kg, i.p.), 5-hydroxytryptophan (5-HTP; 2.5 mg/kg, i.p.), flumazenil (FLU; 8 mg/kg, i.p.) and sodium carboxyl methyl cellulose (CMC-Na) were purchased from Melone Pharma Co. Ltd. (Dalian, China), and diluted in 0.9% physiological saline (Dubang Pharma Co. Ltd., Jilin, China). *p*-Chlorophenylalanine (PCPA, 300 mg/kg, subcutaneously; s.c.) was suspended in 0.5% CMC-Na/physiological saline (Melone Pharma). Diazepam (DZP) (2 mg/kg, intragastically; i.g.) as the positive control was provided by Yimin Pharma Co. Ltd. (Beijing, China), and SFES (10 mg/kg, 25 mg/kg, 50 mg/kg, 100 mg/kg, and 200 mg/kg, i.g.) were administered 30 minutes prior to the locomotor activity test or pentobarbital administration. 5-HTP, FLU, and caffeine were all injected 20 minutes before pentobarbital administration. PCPA was injected 24 hours before administration of SFES to obtain a PCPA-induced model of insomnia. All the drug solutions were freshly prepared before use, and all the tests were carried out between 09:00 and 11:00 hours once daily for four consecutive days.

2.4. HPLC analysis of SFES and SEES

SFES and SEES were analyzed by HPLC [23]. Using an Agilent 1260 LC system (Agilent, Santa Clara, California, USA) equipped with a G1314F VWD detector and an Eclipse XDB-C₁₈ column (4.6 mm $\times$ 250 mm, 5 μm). The column temperature was 30°C , the detector wavelength was set at 220 nm, and the flow rate was 1.0 mL/min. Ultrapure water and acetonitrile were used as Mobile Phase A and B, respectively. The gradient elution was programmed as follows: 0–20 minutes, 50% A; 20–30 minutes, 50–40% A; 30–40 minutes, 45–25% A; 40–50 minutes, 25% A; 50–60 minutes, 25–35% A; 60–65 minutes, 35–50% A.

The mixed reference solution was prepared with acetonitrile at concentrations of 120 $\mu\text{g/mL}$ (schisandrol A), 45 $\mu\text{g/mL}$ (schisandrol B), 30 $\mu\text{g/mL}$ (schisantherin A), 45 $\mu\text{g/mL}$ (schisandrin A), 120 $\mu\text{g/mL}$ (schisandrin B) and 30 $\mu\text{g/mL}$ (schisandrin C). Standard curves of the six lignans were established by six different injection volumes (1 μL , 2 μL , 6 μL , 10 μL , 16 μL , and 20 μL) of the mixed reference solution. The solution was filtered through a 0.45- μm membrane before liquid chromatogram (LC) analysis.

The sample solutions of SFES and SEES were prepared by accurately weighing 0.3 g extract and transferring into a 10-mL volumetric flask with acetonitrile. The solution was filtered through a 0.45- μm membrane before LC. The injection volume of SFES was 5 μL , while the injection volume of SEES was 10 μL .

2.5. Locomotor activity test

The sedative effect of SFES was evaluated by detecting the spontaneous locomotor activity in mice. Locomotor activity and standing could be measured by the ZZ-6 locomotor activity tester (Taimeng Software Co. Ltd., Chengdu, China), which consisted of a microcomputer control system and six separable reaction tanks that possessed 36 points of the infrared array probes. Thirty minutes after oral

administration of SFES and DZP, mice were placed individually in the locomotor activity tester. After adapting for 5 minutes, locomotor activity was recorded for 5 minutes.

2.6. Pentobarbital-induced sleep test

Animals were observed for the onset of sleep, when they were injected with sodium pentobarbital (28 mg/kg or 42 mg/kg, i.p.) after administration of SFES (50 mg/kg, 100 mg/kg, or 200 mg/kg, i.g.) or DZP (2 mg/kg, i.g.) for 30 minutes. When the animals lost their righting reflex over 1 minute, they were considered to be asleep. Sleep latency was recorded from pentobarbital injection to sleep onset; sleep duration was recorded from loss of righting reflex to recovery; and sleep recovery was recorded from recovery of righting reflex to beginning to move.

2.7. Statistical analysis

All values are presented as the mean $\pm$ standard error of the mean. For statistical comparison, data were analyzed by one-way analysis of variance, followed by the Student–Newman–Keuls test. The χ^2 test was used to determine the number of sleeping mice treated by subhypnotic dose of pentobarbital (28 mg/kg). Differences with $p < 0.05$ were considered as statistically significant for all the data.

3. Results

3.1. HPLC analysis of SFES and SEES

All six calibration curves exhibited good linearity in the range of 0.120–2.400 μ g (schisandrol A), 0.045–0.90 μ g (schisandrol B), 0.030–0.60 μ g (schisantherin A), 0.045–0.90 μ g (schisandrin A), 0.120–2.40 μ g (schisandrin B), and 0.030–0.60 μ g (schisandrin C). Linear regression equations and correlation coefficients (R^2) were: $y = 5980.6x - 24.747$ ($R^2 = 1$) (schisandrol A), $y = 11200x - 20.017$ ($R^2 = 1$) (schisandrol B), $y = 8173.6x - 8.6775$ ($R^2 = 1$) (schisantherin A), $y = 9487.4x + 237.95$ ($R^2 = 0.9993$) (schisandrin A), $y = 6497.8x - 10.308$ ($R^2 = 1$) (schisandrin B), and $y = 6623.5x - 15.300$ ($R^2 = 1$) (schisandrin C) (Table 1).

Figure 1 showed the chromatograms of the mixed standards and the SFES and SEES extracts. The content of schisandrol A, schisandrol B, schisantherin A, schisandrin A, schisandrin B, and schisandrin C in SFES and SEES was calculated from their respective standard calibration curves. The

total content of the six lignans in SFES was 33.092 mg/g, while the total content of the six lignans in SEES was 14.192 mg/g. The lignans content was clearly higher in SFES than in SEES.

3.2. Effect of SFES on the reduction of locomotor activity in mice

The sedative activity of SFES was investigated by recording the spontaneous locomotor activity of mice. Compared with the vehicle group, SFES (10 mg/kg, 25 mg/kg, 50 mg/kg, 100 mg/kg, and 200 mg/kg) reduced the locomotor activity of mice dose dependently; the decreased percentage was 7.32%, 12.1%, 15.1%, 17.6%, and 22.7%, respectively. However, SFES (50 mg/kg, 100 mg/kg, and 200 mg/kg) differed significantly compared with the vehicle group ($p < 0.05$) (Table 2). The positive control of DZP (2 mg/kg) significantly decreased the percentage of locomotor activity in mice by 29.5% ($p < 0.01$). Meanwhile, the standing counts that reflected the immobility of mice after i.g. administration of SFES and DZP increased correspondingly.

3.3. Effect of SFES on promotion of pentobarbital-induced sleep in mice

The hypnotic activity of SFES was evaluated by detecting the synergistic effect with sodium pentobarbital in mice. SFES (50 mg/kg, 100 mg/kg, and 200 mg/kg) augmented the hypnotic effect of pentobarbital (42 mg/kg) significantly with prolonging sleep time ($p < 0.01$, Figure 2B), decreasing sleep latency ($p < 0.01$, Figure 2A) and sleep recovery ($p < 0.05$, Figure 2C). SFES also increased the number of mice falling asleep induced by subhypnotic dose of pentobarbital (28 mg/kg) in a dose-dependent manner ($p < 0.05$ or $p < 0.01$) (Table 2).

The spontaneous locomotor activity was assessed for the sedative effect of SFES. Values are presented as mean $\pm$ standard error of the mean ($n = 10$ in each group), * $p < 0.05$, ** $p < 0.01$ versus vehicle group. Mice were pretreated with CMC-Na/physiological saline, DZP and SFES 30 minutes prior to injection of a subhypnotic dose of pentobarbital (28 mg/kg). The number of mice falling asleep was recorded (# $p < 0.05$, ## $p < 0.01$ vs. vehicle group, χ^2 test).

3.4. Effect of SFES on reversion of caffeine-induced insomnia in mice

Caffeine-treated mice have been used as a model of insomnia [24]. This study showed that the sleep latency and sleep recovery time of mice treated with caffeine (7.5 mg/kg, i.p.)

Table 1 – Linearity calibration curves of the six lignans and their content in SFES, SEES.

Compounds	Retention time (min)	Standard curve	R^2	Liner range (μ g)	Content in SFES (mg/g)	Content in SEES (mg/g)
Schisandrol A	9.661	$y = 5980.6x - 24.747$	1.0000	0.120–2.400	11.781	6.872
Schisandrol B	12.802	$y = 11200x - 20.017$	1.0000	0.045–0.900	3.775	1.885
Schisantherin A	29.906	$y = 8173.6x - 8.6775$	1.0000	0.030–0.600	1.116	0.399
Schisandrin A	42.126	$y = 9487.4x + 237.95$	0.9993	0.045–0.900	1.213	0.438
Schisandrin B	45.945	$y = 6497.8x - 10.308$	1.0000	0.120–2.400	11.910	3.750
Schisandrin C	48.321	$y = 6623.5x - 15.300$	1.0000	0.030–0.600	3.297	0.848

SEES = the ethanol extract of *Schisandra chinensis*; SFES = superfluid extract of *Schisandra chinensis*.

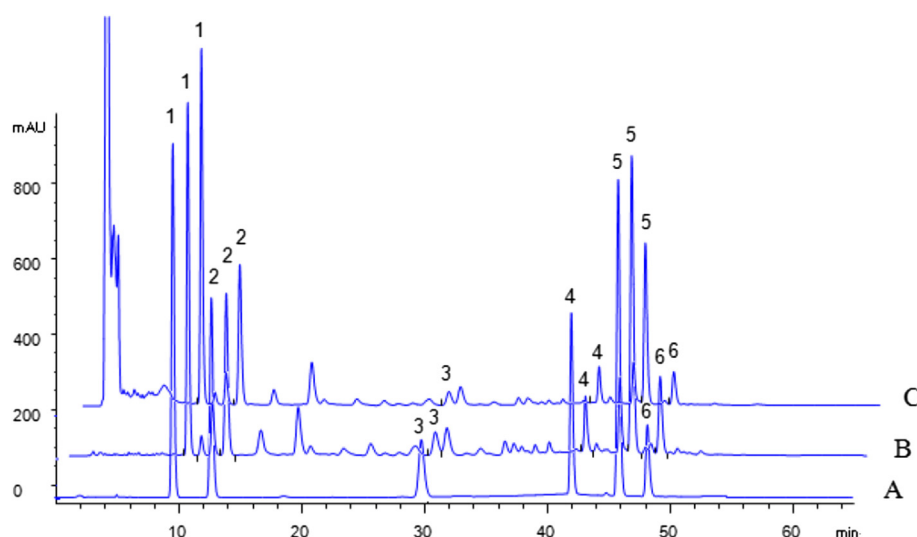


Figure 1 – HPLC of reference compounds (A), sample of SFES (B), and SEES (C). 1 = Schisandrol A; 2 = Schisandrol B; 3 = Schisantherin A; 4 = Schisandrin A; 5 = Schisandrin B; 6 = Schisandrin C. SEES = the ethanol extract of *Schisandra chinensis*; SFES = superfluid extract of *Schisandra chinensis*.

20 minutes after injection of sodium pentobarbital (42 mg/kg, i.p.) significantly increased ($p < 0.01$, Figures 2A and 2C), and the sleeping time decreased ($p < 0.05$ or $p < 0.01$, Figure 2B) compared with the normal groups. SFES (50 mg/kg, 100 mg/kg, and 200 mg/kg) significantly reversed insomnia in caffeine-treated mice by decreasing sleep latency and sleep recovery ($p < 0.05$ or $p < 0.01$, Figures 2A and 2C), and increasing the sleeping time significantly ($p < 0.01$, Figure 2B). In addition, the effects of SFES represented a dose-dependent effect.

3.5. Effect of SFES on reversion of PCPA-induced insomnia in pentobarbital-treated mice

As described previously [25], PCPA (300 mg/kg) as an inhibitor of tryptophan hydroxylase can induce insomnia. This study showed that pretreatment with PCPA (300 mg/kg, s.c.) 24 hours before pentobarbital (42 mg/kg, i.p.) injection significantly increased sleep latency and sleep recovery ($p < 0.01$, Figures 3A and 3C) and decreased sleep time ($p < 0.01$, Figure 3B) in the vehicle group. The SFES-treated groups significantly reversed the PCPA-induced insomnia in

pentobarbital-treated mice by increasing sleeping time at a dose of 50 mg/kg ($p < 0.05$), 100 mg/kg ($p < 0.01$), and 200 mg/kg ($p < 0.01$) (Figure 3B), and reducing sleep latency at a dose of 50 mg/kg ($p < 0.05$), 100 mg/kg ($p < 0.01$), and 200 mg/kg ($p < 0.01$) (Figure 3A). Meanwhile, sleep recovery was also decreased ($p < 0.05$, Figure 3C).

3.6. Synergistic effect of SFES and 5-HTP in pentobarbital-treated mice

Mice were pretreated with 5-HTP (2.5 mg/kg) 20 minutes prior to injection of pentobarbital (42 mg/kg, i.p.) to investigate the relationship between the hypnotic activity of SFES and the serotonergic system (Figure 4). Neither SFES (10 mg/kg) nor 5-HTP (2.5 mg/kg) administered individually affected sleep latency or sleep duration. While the synergistic effect of SFES (10 mg/kg) and 5-HTP (2.5 mg/kg) significantly prolonged sleeping time ($p < 0.01$, Figure 4B) and shortened sleep latency ($p < 0.05$, Figure 4A), sleep recovery decreased but there was no significant difference compared with the control group (Figure 4C).

3.7. Effect of SFES on reversion of FLU-induced insomnia in pentobarbital-treated mice

FLU antagonizes the benzodiazepine (BZD) site of the γ -aminobutyric acid (GABA)_A receptor, and inhibits sedative and hypnotic drugs such as DZP from binding to GABA_A-BZD receptors [26,27]. The synergistic effect of SFES and FLU was investigated to find the relationship of SFES with the GABAergic system. Mice were pretreated with FLU (8 mg/kg, i.p.) 20 minutes prior to injection of pentobarbital (42 mg/kg, i.p.). SFES-induced decreasing of sleep latency ($p < 0.01$, Figure 3A), sleep recovery ($p < 0.05$, Figure 3C) and increased sleep time ($p < 0.01$, Figure 3B) were also found to be antagonized by FLU.

Table 2 – Effect of SFES on spontaneous locomotor activity in normal mice and sleep onset of mice treated with subhypnotic dose of pentobarbital (28 mg/kg).

Group	Dosage (mg/kg, i.g.)	Locomotion counts in 5 min	No. falling asleep/total	Sleep onset (%)
Vehicle	—	87.4 ± 5.5	0/10	0
DZP	2	61.6 ± 16.4**	10/10	100**
SFES	50	74.2 ± 7.6*	2/10	20#
	100	72.0 ± 10.3*	6/10	60**
	200	67.6 ± 16.2*	7/10	70**

DZP = diazepam; i.g. = intragastric; SFES = superfluid extract of *Schisandra chinensis*. By chi-square test (The χ^2 test), DZP and SFES treated-mice vs. the vehicle group.

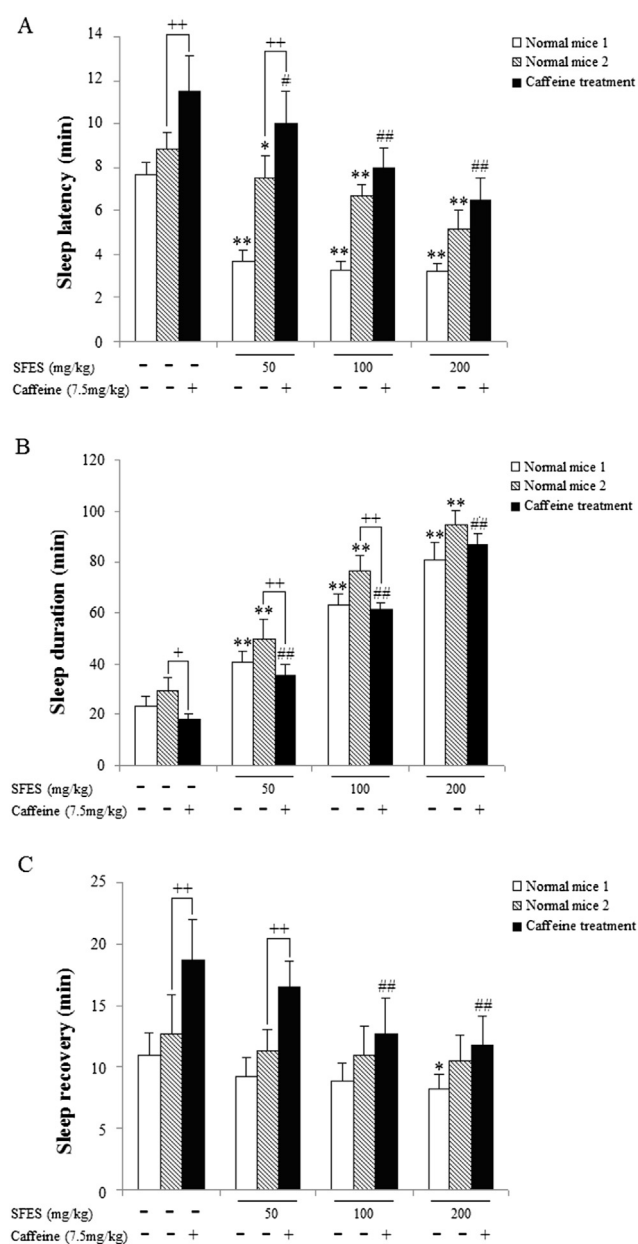


Figure 2 – Effect of SFES on pentobarbital-treated sleep in normal mice 1, and the effect on caffeine-induced insomnia mice, to which caffeine (7.5 mg/kg, i.p.) was given 20 minutes before administration. All groups were treated with pentobarbital (42 mg/kg, i.p.) 30 minutes after the last administration. Sleep latency (A), sleep duration (B), and sleep recovery (C) were assessed. All values are presented as mean $\pm$ standard error of the mean ($n = 10$ in each group). * $p < 0.05$, ** $p < 0.01$ versus normal mice 1 or normal mice 2; # $p < 0.05$, ## $p < 0.01$ versus caffeine-treated group; + $p < 0.05$, ++ $p < 0.01$ caffeine-treated groups versus normal mice 2. i.p. = intraperitoneally; SEES = the ethanol extract of *Schisandra chinensis*; SFES = superfluid extract of *Schisandra chinensis*.

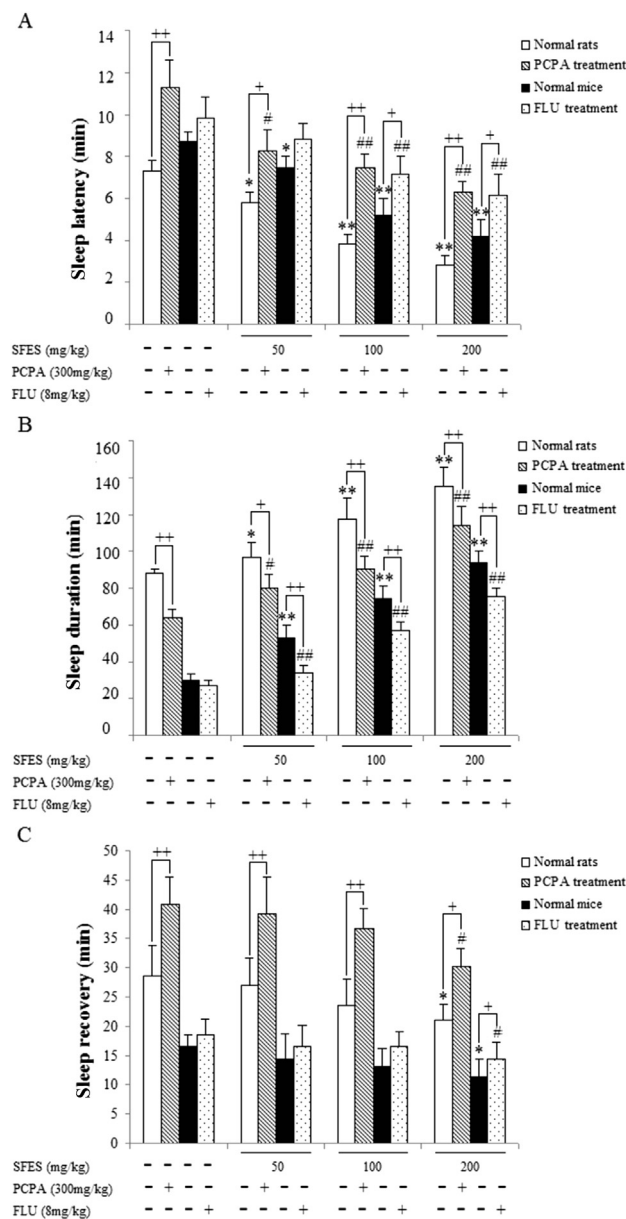


Figure 3 – Reversion effects of SFES on PCPA-induced insomnia in pentobarbital-treated rats, which were pretreated with PCPA (300 mg/kg, s.c.) 24 hours prior to injection of pentobarbital (42 mg/kg, i.p.), and the effect on FLU-induced sleep disturbance in pentobarbital-treated mice, which were pretreated with FLU (8 mg/kg, i.p.) 20 minutes prior to injection of pentobarbital (42 mg/kg, i.p.). Sleep latency (A), sleep duration (B), and sleep recovery (C) were assessed. All values are presented as mean $\pm$ standard error of the mean ($n = 10$ in each group) * $p < 0.05$, ** $p < 0.01$ versus vehicle normal rats or normal mice; # $p < 0.05$, ## $p < 0.01$ versus vehicle model group induced by PCPA or FLU; + $p < 0.05$, ++ $p < 0.01$ model groups versus normal groups. FLU = flumazenil; i.p. = intraperitoneally; PCPA = *p*-chlorophenylalanine; s.c. = subcutaneously; SFES = superfluid extract of *Schisandra chinensis*.

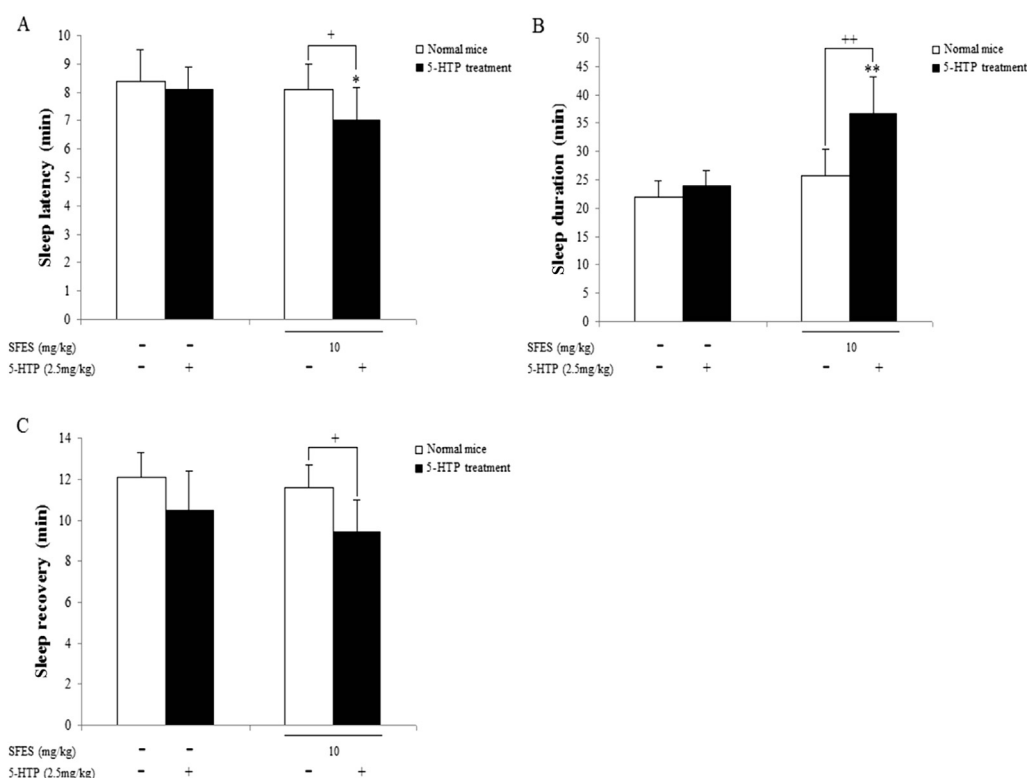


Figure 4 – Synergistic effects of SFES with 5-HTP on pentobarbital-treated sleep in mice. Mice are pretreated with 5-HTP (2.5 mg/kg, i.p.) 20 minutes prior to injection of pentobarbital (42 mg/kg, i.p.). Sleep latency (A), sleep duration (B), and sleep recovery (C) were assessed. All values are presented as the mean $\pm$ standard error of the mean ($n = 10$ in each group). * $p < 0.05$, ** $p < 0.01$ versus vehicle model group treated with 5-HTP; + $p < 0.05$, ++ $p < 0.01$ model groups versus normal groups. 5-HTP = 5-hydroxytryptophan; i.p. = intraperitoneally; SFES = superfluid extract of *Schisandra chinensis*.

4. Discussion

S. chinensis, alone or in formula, has been used for treatment of insomnia for a long time. Lignans are the main active components, such as schisandrol A, schisandrol B, schisandrin A, and schisandrin B. There are many methods for extraction and purification of lignans from *S. chinensis*, such as ion exchange resin transformation [28,29], homogeneous ionic liquid microextraction [30], ionic liquid-based ultrasound-assisted extraction [31], ultrasound extraction [32–34], and supercritical fluid extraction [20]. Supercritical carbon dioxide that is free of organic solvents was used to extract the lignans of *S. chinensis* in this study. Our previous results showed that there were >58% of lignans in the SFES by gas chromatography–mass spectrometer (GC-MS) analysis, which was more than ethanol extraction of *S. sphenanthera* (33% lignans) [18]. Meanwhile, HPLC analysis of the six representative lignans (schisandrol A, schisandrol B, schisantherin A, schisandrin A, schisandrin B, and schisandrin C) in SFES and SEES also showed that there were more lignans in SFES than SEES in the same quality of extracts, although the extraction ratio of SFES was distinctly less than SEES.

To investigate the sedative and hypnotic effects of SFES, the locomotor activity and pentobarbital-induced sleep test in mice were conducted, which were the two classic behavioral

pharmacology methods for evaluating sedative–hypnotic activity, because sleep as a sophisticated physiological process is affected by a network of neuronal systems [35], which is hardly reproduced *in vitro*. The decrease of spontaneous locomotion was considered to be sedative as it reduced excitability of the central nervous system [36]. The synergic effects of SFES and pentobarbital on mouse sleep, in which sleep latency, duration, and recovery were recorded, meanwhile the effect of SFES on caffeine-induced insomnia, which was a simple and effective model of insomnia [24], were performed in this study to detect the hypnotic activity of SFES. DZP as a typical sedative reagent was chosen to be the positive control.

SFES significantly reduced the locomotor activity of mice compared with the vehicle group in a dose-dependent manner. The sleep latency was significantly shortened (Figure 2A), the sleeping time was prolonged (Figure 2B), and the sleep recovery, which could reflect the duration of effect on the central nervous system after waking, was shortened at both doses, but only the highest dose revealed a significant difference (Figure 2C). All the effects were dose dependent. In the caffeine-induced insomnia test (Figure 2), SFES had a counteractive effect on the reduced sleep latency and increased sleep duration, while sleep recovery had little change. The above results indicate that SFES could accelerate sleep onset, prolong sleeping time, and promote waking from sleep in mice, and showed excellent hypnotic activity. Most

evaluation of the hypnotic effects of pentobarbital-induced sleep tests only selected sleep latency and duration as an index [37,38]. Sleep recovery, which was recorded from the recovery of righting reflex to beginning of movement, reflected the subsequent effect on the central nervous system. Good potential hypnotics should not affect awakening after sleep, and SFES showed this feature, which might have been due to the dual regulatory effects of traditional Chinese medicine [39].

Pharmacokinetic studies of ethanol extraction of *S. chinensis* have revealed that the hypnotic activity might be related to increased level of serotonin and 5-hydroxyindoleacetic acid, and the decreased level of GABA, norepinephrine (NE), dopamine (DA), dihydroxy-phenylalanine (DOPA), and homovanillic acid (HVA) [40]. The six lignans schisandrin, schisandrol B, schisantherin A, deoxyschisandrin, γ -schisandrin, and gomisins N might be the active components [15]. Behavioral pharmacological tests on mice or rats about the influence of the serotonergic, noradrenergic, dopaminergic, or GABAergic systems have never been reported. The “serotonin sleep hypothesis” shows that serotonin plays an important role in modulation of sleep [41,42], as the inhibition of serotonin synthesis by PCPA induced insomnia, and treatment with 5-HTP, a serotonin precursor, induced drowsiness. In order to research whether the hypnotic effect of SFES was based on the serotonergic system, we carried out experiments with the PCPA/5-HTP model. The results showed that SFES reversed insomnia in rats with pretreatment of PCPA (Figure 3), and had synergistic effects with subhypnotic doses of 5-HTP on pentobarbital-induced sleep in mice (Figure 4). This suggests that the serotonergic system might be involved in the hypnotic mechanism of SFES on pentobarbital-induced hypnosis.

GABA is an important inhibitory neurotransmitter in the brain [43], and a lot of sedative–hypnotic drugs such as DZP, focus on GABA_A-BZD receptor binding [26]. To investigate whether SFES had potential hypnotic effects through the GABAergic system, the effects of coadministration of SFES and FLU, a specific BZD antagonist, were investigated [44]. All doses of SFES could be inhibited by FLU (Figure 3), indicating that SFES acted at the GABA_A-BZD receptors similar to DZP, so the hypnotic effect of SFES was attributed to the modulation of GABA_A-BZD receptors through the GABAergic system.

Our previous analysis of SFES by GC-MS showed that there were >58% of lignans in which schisandrol A and schisandrin B comprised the majority (data not shown). The sedative–hypnotic effect of schisandrin was reported to be related to the serotonergic system but was not influenced by FLU [45]. Further studies are needed to investigate whether the modification of serotonergic system was attributed to schisandrin alone or with other lignans, and whether the modification of GABAergic system belonged to schisandrin B or other lignans. The precise mechanism by which subtypes of the 5-HT receptor are related to the serotonergic system also needs further experimentation.

Conflicts of interest

No potential conflict of interest was reported by the authors.

Acknowledgments

This work received financial support from the National Key Technology Support Program (No. 2011BAI03B010602), National Scientific and Technology Major Project (No. 2012ZX090304006), National Public Scientific Research (No. 201303111), Science and Technology Development Program of Jilin Province (No. 20126046), World Bank loan to Jilin Agricultural Product Quality Safety (No. 2011-Z25), and Development Plan of Science and Technology of Jilin Province (No. 20130102075JC).

REFERENCES

- [1] Committee of National Pharmacopoeia. Pharmacopoeia of P.R. China. Beijing: Chinese Medical Science and Technology Press; 2010. p. 61–2 [in Chinese].
- [2] Dai HF, Zhou J, Peng ZG, Tan NH, Kurihara H. Studies of the chemical constituents of *Schisandra chinensis*. Nat Prod Res Dev 2001;13:24–6.
- [3] Dai HF, Tan NH, Zhou J. Glycosides from *Schisandra chinensis*. Chin J Nat Med 2006;4:49–51 [in Chinese].
- [4] Casarin E, Dall'Acqua S, Smejkal K, Šlapetová T, Innocenti G, Carrara M. Molecular mechanisms of antiproliferative effects induced by *Schisandra*-derived dibenzocyclooctadiene lignans (+)-deoxyschisandrin and (–)-gomisin N in human tumour cell lines. Fitoterapia 2014;98:241–7.
- [5] Chen WW, He RR, Li FY, Li SB, Tsoi B. Pharmacological studies on the anxiolytic effect of standardized *Schisandra* lignans extract on restraint-stressed mice. Phytomed 2011;18:1144–7.
- [6] Guo LY, Hung TM, Bae KH, Shin EM, Zhou HY, Hong YN, Kang SS, Kim HP, Kim YS. Anti-inflammatory effects of schisandrin isolated from the fruit of *Schisandra chinensis* Baill. Eur J Pharmacol 2008;591:293–9.
- [7] Kim DH, Hung TM, Bae KH, Jung JW, Lee S, Yoon BH, Cheong JH, Ko KH, Ryu JH. Gomisins A improves scopolamine-induced memory impairment in mice. Eur J Pharmacol 2006;542:129–35.
- [8] Lee HJ, Kim CY. Simultaneous determination of nine lignans using pressurized liquid extraction and HPLC-DAD in the fruits of *Schisandra chinensis*. Food Chem 2010;120:1224–8.
- [9] Sun M, Xu XL, Lu QH, Pan QR, Hu X. Schisandrin B: a dual inhibitor of P-glycoprotein and multidrug resistance-associated protein 1. Cancer Lett 2007;246:300–7.
- [10] Takimoto Y, Qian HY, Yoshigai E, Okumura T, Ikeya Y, Nishizawa M. Gomisins N in the herbal drug gomishi (*Schisandra chinensis*) suppresses inducible nitric oxide synthase gene via C/EBP β and NF- κ B in rat hepatocytes. Nitric Oxide 2014;28:47–56.
- [11] Tang SH, He RR, Huang T, Wang CZ, Cao YF, Zhang Y, Kurihara H. The protective effect of *Schisandra* lignans on stress-evoked hepatic metastases of P815 tumor cells in restraint mice. J Ethnopharmacol 2011;134:141–6.
- [12] Wan CK, Zhu GY, Shen XL, Chattopadhyay A, Dey S, Fong WF. Gomisins A alters substrate interaction and reverses P-glycoprotein-mediated multidrug resistance in HepG2-DR cells. Biochem Pharmacol 2006;72:824–37.
- [13] Wan CK, Tse AK, Yu ZL, Zhu GY, Wang H, Fong DWF. Inhibition of cytochrome P450 3A4 activity by schisandrol A and gomisins A isolated from *Fructus Schisandra chinensis*. Phytomed 2010;17:702–5.
- [14] Wang XX, Hu D, Zhang LJ, Lian GN, Zhao SQ, Wang CM, Yin J, Wu CF, Yang JY. Gomisins A inhibits lipopolysaccharide-

- induced inflammatory responses in N9 microglia via blocking the NF- κ B/MAPKs pathway. *Food Chem Toxicol* 2014;63:119–27.
- [15] Wei BB, Li Q, Su D, Fan RH, Zhao LS, Geng LL, He BS, Chen XH, Jia Y, Bi KS. Development of a UFLC-MS/MS method for simultaneous determination of six lignans of *Schisandra chinensis* (Turcz.) Baill. In rat plasma and its application to a comparative pharmacokinetic study in normal and insomnic rats. *J Pharmaceut Biomed Anal* 2013;77:120–7.
 - [16] Zhang CN, Mao X, Zhao X, Liu Z, Liu B, Li H, Bi KS, Jia Y. Gomisin N isolated from *Schisandra chinensis* augments pentobarbital-induced sleep behaviors through the modification of the serotonergic and GABAergic system. *Fitoterapia* 2014;96:123–30.
 - [17] Lu Y, Chen DF. Analysis of *Schisandra chinensis* and *Schisandra sphenanthera*. *J Chromatogr A* 2009;1216:1980–90.
 - [18] Huang F, Xiong YT, Xu LH, Ma SP, Dou CG. Sedative and hypnotic activities of the ethanol fraction from *Fructus Schisandrae* in mice and rats. *J Ethnopharmacol* 2007;110:471–5.
 - [19] Choi YW, Takamatsu S, Khan SI, Srinivas PV, Ferreira D, Zhao JP, Khan IA. Schisandrene, a dibenzocyclooctadiene lignan from *Schisandra chinensis*: structure- antioxidant activity relationships of dibenzocyclooctadiene lignans. *J Nat Prod* 2006;69:356–9.
 - [20] Lin CH, Lin HW, Wu JY, Hwang JY, Wan HP, Yang TY, Liang MT. Extraction of Lignans from the Seed of *Schisandra chinensis* by supercritical fluid extraction and subsequent separation by supercritical fluid simulated moving bed. *J Supercrit Fluid* 2015;98:17–24.
 - [21] Slanina J, Táborská E, Lojková L. Lignans in the seeds and fruits of *Schisandra chinensis* cultured in Europe. *Planta Medica* 1997;63:277–80.
 - [22] Sovová H, Opletal L, Bártlová M, Sajfrtová M, Křenková M. Supercritical fluid extraction of lignans and cinnamic acid from *Schisandra chinensis*. *J Supercrit Fluid* 2007;42:88–95.
 - [23] Wang GL, Zhu HY, Lin HC, Chi K, Yao YY, Zhang LX. Simultaneous determination of six lignans in *Schisandra chinensis* from different samples by reverse phase high-performance liquid chromatography. *J Food Safety Quality* 2014;5:2470–5 [in Chinese].
 - [24] Paterson LM, Wilson SJ, Nutt DJ, Hutson PH, Ivarsson M. A translational, caffeine-induced model of onset insomnia in rats and healthy volunteers. *Psychopharmacol* 2007;191:943–50.
 - [25] Borbély AA, Neuhaus HU, Tobler I. Effect of *p*-chlorophenylalanine and tryptophan on sleep, EEG and motor activity in the rat. *Behav Brain Res* 1981;2:1–22.
 - [26] Bateson AN. Further potential of the GABA receptor in the treatment of insomnia. *Sleep Med* 2006;7S1:S3–9.
 - [27] Ma H, Jo YJ, Ma Y, Hong JT, Kwon BM, Oh KW. Obovatol isolated from *Magnolia obovata* enhances pentobarbital-induced sleeping time: Possible involvement of GABA_A receptors/chloride channel activation. *Phytomed* 2009;16:308–13.
 - [28] Ma CH, Yang L, Zu YG, Wang NH, Zhang L, Zhang Y, Chen XQ, Zhao CJ. A new approach to catalytic hydrolysis of ester-bound biphenyl cyclooctene lignans from the fruit of *Schisandra chinensis* Baill by ion exchange resin. *Chem Eng Res Des* 2012;90:1189–96.
 - [29] Ma CH, Liu TT, Yang L, Zu YG, Yang FJ, Zhao CJ, Zhang L, Zhang ZH. Preparation of high purity biphenyl cyclooctene lignans from *Schisandra* extract by ion exchange resin catalytic transformation combined with macroporous resin separation. *J Chromatogr B* 2011;879:3444–51.
 - [30] Xiao Y, Zhang HQ. Homogeneous ionic liquid microextraction of the active constituents from fruits of *Schisandra chinensis* and *Schisandra sphenanthera*. *Analytica Chimica Acta* 2012;712:78–84.
 - [31] Ma CH, Liu TT, Yang L, Zu YG, Wang SY, Zhang RR. Study on ionic liquid-based ultrasonic-assisted extraction of biphenyl cyclooctene lignans from the fruit of *Schisandra chinensis* Baill. *Analytica Chimica Acta* 2011;689:110–6.
 - [32] Zhang YB, Wang LH, Zhang DY, Zhou LL, Guo YX. Ultrasound-assisted extraction and purification of schisandrin B from *Schisandra chinensis* (Turcz.) Baill seeds: optimization by response surface methodology. *Ultrason Sonochem* 2014;21:461–6.
 - [33] Guo YX, Han J, Zhang DY, Wang LH, Zhou LI. Aqueous two-phase system coupled with ultrasound for the extraction of lignans from seeds of *Schisandra chinensis* (Turcz.) Baill. *Ultrason Sonochem* 2013;20:125–32.
 - [34] Cheng ZY, Yang YJ, Liu Y, Liu ZG, Zhou HL, Hu HB. Two-steps extraction of essential oil, polysaccharides and biphenyl cyclooctene lignans from *Schisandra chinensis* Baill fruits. *J Pharmaceut Biomed Anal* 2014;96:162–9.
 - [35] Fuller PM, Gooley JJ, Saper CB. Neurobiology of the sleep-wake cycle: sleep architecture, circadian regulation, and regulatory feedback. *J Biol Rhythm* 2006;21:482–93.
 - [36] Öztürk Y, Aydın S, Beis R, Baser KHC, Berberoğlu H. Effects of *Hypericum perforatum* L. and *Hypericum calycinum* L. extracts on the central nervous system in mice. *Phytomed* 1996;3:139–46.
 - [37] Cho SM, Shimizu M, Lee CJ, Han DS, Jung CK, Jo JH, Kim YM. Hypnotic effects and binding studies for GABA_A and 5-HT_{2C} receptors of traditional medicinal plants used in Asia for insomnia. *J Ethnopharmacol* 2010;132:225–32.
 - [38] Wu XY, Zhao JL, Zhang M, Li F, Zhao T, Yang LQ. Sedative, hypnotic and anticonvulsant activities of the ethanol fraction from *Rhizoma Pinelliae* Praeparatum. *J Ethnopharmacol* 2011;135:325–9.
 - [39] Wang BX. Pharmacology of *Fructus Schisandrae chinensis*. *J Tianjin Med* 1965;7:338–44.
 - [40] Wei BB, Li Q, Fan RH, Su D, Chen XH, Jia Y, Bi KS. Determination of monoamine and amino acid neurotransmitters and their metabolites in rat brain samples by UFLC-MS/MS for the study of the sedative-hypnotic effects observed during treatment with *S. chinensis*. *J Pharmaceut Biomed Anal* 2014;88:416–22.
 - [41] Dugovic C. Role of serotonin in sleep mechanisms. *Revue Neurologique* 2001;157:S16–9.
 - [42] Ursin R. Serotonin and sleep. *Sleep Med Rev* 2002;6:55–67.
 - [43] Yang RQ, Guo YX, Wang SF, Gu ZX. Ca²⁺ and aminoguanidine on γ -aminobutyric acid accumulation in germinating soybean under hypoxia-NaCl stress. *J Food Drug Anal* 2015;23:287–93.
 - [44] Johnston GAR. GABA_A receptor channel pharmacology. *Curr Pharm Design* 2005;11:1867–85.
 - [45] Zhang CN, Zhao X, Mao X, Liu AJ, Liu Z, Li XL, Bi KS, Jia Y. Pharmacological evaluation of sedative and hypnotic effects of schisandrin through the modification of pentobarbital-induced sleep behaviors in mice. *Eur J Pharmacol* 2014;744:157–63.