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Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com**Original article****Research on Hypnotic and Anticonvulsant Activities of Total Alkaloids in Leaves of *Eucommia ulmoides***Xin Li^{1*}, Li-peng Yang¹, Xin-ling Li¹, Li-ping Wang¹, Shao-yang Liu², Yan Liu³

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ABSTRACT

Objective To study the hypnotic and anticonvulsant activities of the leaves of *Eucommia ulmoides* (LEU), the total alkaloids were extracted from LEU using water-acid method. **Methods** Mice were divided into five groups, including groups contained total alkaloids in three different doses, negative and positive control groups. Direct hypnotic experiment in mice, pentobarbital sodium synergistic experiment, and anticonvulsant experiment were used. The numbers of spontaneous activities, sleep rate, sleep latency, sleep time, convulsion rate, and convulsion latency of the mice were recorded and analyzed. **Results** The total alkaloids from LEU could increase the sleep rate, significantly lengthen the sleep time, and shorten the sleep latency of mice. Even the low dose of total alkaloids (0.33 g/kg) showed significantly different activities with negative control group (physiological saline) with the synergistic effect of the superthreshold dose of pentobarbital sodium. Furthermore, the total alkaloids efficiently inhibited the convulsion caused by nikethamide. **Conclusion** The total alkaloids from LEU have the excellent sedative, hypnotic, and anticonvulsant activities in mice, with high safety and little drug side effects. Therefore, they have the potential development prospects in sedative-hypnotic drugs.

*Key words**Eucommia ulmoides*; hypnotic effect; sedative effect; total alkaloids

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1. Introduction

Insomnia is a common sleep disorder in clinic, with a complicated pathogenesis, which is very likely to relapse. At present, sedately hypnotic agents (Western medicine) are conventionally used to treat insomnia both in China and abroad, but all of these medicines have side effects and could cause drug dependence to some extent (Dou and Zhao, 2004). Some Chinese materia medica (CMM) have excellent hypnotic effects (Vaninac and Valdemar, 2009; Wang and

Chen, 2012; Li et al, 2012) without causing side effects or drug dependence. The hypnotic effects of CMM have recently become a hot research topic.

Eucommia ulmoides Oliver is a precious nourishing supplement in CMM, and modern medical research has proven that it had the following functions: regulating blood pressure and blood fat, reducing blood sugar, antibacterial effect, diminishing inflammation, inhibiting tumor cell growth, nourishing *Yin* and invigorating the kidney, strengthening muscles and bones, delaying aging, etc (Liu and Liu, 2002).

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Recent studies have indicated that all the seeds, leaves, and flowers of *E. ulmoides* had hypnotic effects (Cheng, 2006; Lian et al, 2007; Guan and Su, 2003). However, the specific active ingredients in *E. ulmoides* and their mechanism were still not clear.

At present, lots of studies on the function and activity of *E. ulmoides* have been reported (Zhan et al, 2001; Zhao and Zhang, 2003; Lian et al, 2007). An earlier study in this laboratory showed that the leaves of *Eucommia ulmoides* (LEU) had the excellent hypnotic effects, but the active ingredients in LEU have not been clearly clarified either in China or foreign countries. Meanwhile, relevant studies have found that total alkaloids had multiple physiological functions, and the hypnotic and anticonvulsant effects were especially significant (Geng and Xu, 2007; Fu et al, 2005). But there are no relevant reports to confirm whether the total alkaloids in LEU have the hypnotic and anticonvulsant effects, or whether the total alkaloids from LEU are the active ingredients that produce the hypnotic and anticonvulsant effects.

This study was an initial investigation to examine the hypnotic and anticonvulsant activities of the total alkaloids in LEU, and to provide a theoretical basis for the further studies about the hypnotic and anti-convulsant active ingredients of LEU as well as the corresponding mechanism. This study also aimed at providing the support for the development of new, safe, and reliable hypnotic drugs.

2. Materials and methods

2.1 Materials

The leaves of *Eucommia ulmoides* Oliver were picked from the plantation base of Wangping town, Ruyang county, Luoyang city. The plant was identified by Prof. Zhong-dong Wang (College of Chemical Engineering & Pharmaceutics, Henan University of Science and Technology, Luoyang, China) and the voucher specimen (No. Euc-20090603) has been deposited in our laboratory.

Male Kunming mice (20–30 g) of ordinal level were provided by Animal Test Center of Henan Science and Technology University, license No. SCXK (Yu) 2005-0001.

Test reagents for total alkaloids were bismuth potassium iodide, iodide-potassium iodide, and silicotungstic acid, and these reagents were all prepared in the laboratory. Petroleum ether, chloroform, sodium hydroxide, and *n*-butyl alcohol produced by Shanghai Shiyi Chemical Reagent Co., Ltd. were all analytically pure; The pentobarbital sodium was produced by Sigma Company and packaged in Shanghai; Nikethamide, 0.9% sodium chloride solution, and diazepam were produced by Shanghai Shiyi Chemical Reagent Co., Ltd. GJ-1 type Photoelectric Counting Instrument was purchased from Tianjin Medical Device Repair Factory.

2.2 Preparation of total alkaloids

LEU were crushed into small pieces, and then dried using vacuum drying method after petroleum ether extraction.

The leaves (1.5 kg) were taken into 1.5% hydrochloric acid-water solution (1:5, pH 2–3) and soaked for four times (2 d for the first time, then 1 d, 12 h, and 6 h). Then the lipids were washed off with chloroform, the pH value was adjusted to 10–11 with sodium hydroxide, and the precipitation and alkali water solution were obtained. This precipitation was extracted and concentrated with chloroform, respectively, and the fat soluble total alkaloids were obtained using the reduced pressure distillation method. The pH value of the alkali water solution (after extraction of fat soluble total alkaloids) was adjusted to 6.5–7.0, then soaked in *n*-butyl alcohol for 24 h, extracted for three times, and concentrated by reduced pressure distillation method to obtain the water soluble total alkaloids. The water soluble and fat soluble total alkaloids were mixed to obtain the total alkaloids of LEU (Yu and Wang, 2006; Li et al, 2007; Michael, 2007; Talaty et al, 2005).

2.3 Direct sedative and hypnotic effects

2.3.1 Animal grouping

The mice were put in the activity box with a GJ-1-Type Photoelectric Counting Instrument before the experiment. The activity number within 5 min was recorded. The mice that exhibited about 150 times of spontaneous activities were selected. Then, the mice were assigned to experimental groups according to the number of spontaneous activities. There were no apparent differences among the mice assigned to different groups before the experiment.

2.3.2 Experiment grouping

Fifty Kunming mice were divided into five groups, 10 in each group, such as saline-negative control, high-, mid-, and low-dose total alkaloids, and Diazepam-positive control groups. After 30 min of the last administration, the number of spontaneous activities within 5 min was measured. Meanwhile, the status of the mice was carefully observed, and the sedation rate and sleep rate within 30 min were recorded. The drug was regarded as having sedative effect if the mice rested for more than 15 s.

2.4 Sedative effect of pentobarbital sodium in subthreshold dose

2.4.1 Threshold dose experiment

Forty mice were divided into four groups, 10 mice in each group. The mice in each group were ip administered with pentobarbital sodium at the doses of 25, 30, 35, and 40 mg/kg. The sleep rate within 30 min was recorded (Zhang et al, 2007).

2.4.2 Subthreshold dose experiment

The mice in the saline-negative control group were ig administered with physiological saline; The mice in the Diazepam-positive control group were administered with 0.30% Diazepam (5 mg/kg) (Fu et al, 2005; Li et al, 2007); The mice in the high-, mid-, and low-dose total alkaloids

groups were ig administered at the doses of 1.0, 0.5, and 0.33 g/kg, respectively. After 60 min of the last administration, the mice in each group received an injection of pentobarbital sodium at the subthreshold dose (25 mg/kg). The sleep rate within 30 min, sleep latency (from injection of pentobarbital sodium to abolition of righting reflex), and sleep time were recorded. Abolition of righting reflex for more than 1 min was regarded as falling asleep. And the sleep time was the time from righting reflex disappearance to righting reflex recovery.

2.5 Sedative effect of pentobarbital sodium at superthreshold dose

The mice were administered by the same method as "2.4.2". After 60 min of the last administration, the mice in each group received an ip injection of pentobarbital sodium (40 mg/kg, superthreshold dose). The sleep latency and sleep time were recorded.

2.6 Anticonvulsant activities

The mice were administered by the same method as "2.4.2". After 60 min of the last administration, the mice in each group received an ip injection of 1.25% nikethamide (0.01 mL/g) (Frank and Jhamadnas, 1970). After 30 min of treatment, the convulsion latency and convulsion rate were recorded (The systematic tonic convulsion was considered as an index).

2.7 Statistical analysis

The statistical software SPSS 11.0.1 was used to analyze the data. After ig administration of total alkaloids in LEU at the different doses, single factor variance analysis was used to analyze the resulting sedative effect in mice. The paired sample *t*-test was used to analyze the differences between the total alkaloids groups and control groups.

3. Results

3.1 Examination and identification of total alkaloids

Bismuth potassium iodide test, iodide-potassium iodide test, and silicotungstic acid test were performed to confirm the total alkaloids in LEU. The phenomenon that precipitation was produced after the addition of any one of the three

reagents indicated that the LEU contained total alkaloids. The yield rate of total alkaloids in LEU was 3.13%. The quantities of the total alkaloids extracted by chloroform and *n*-butyl alcohol were 8.72 and 36.18 g, respectively, accounting for 19.42% and 80.58% of the total alkaloids. The total alkaloids were mainly from the *n*-butyl alcohol extraction, which indicated that most of the total alkaloids in LEU were large polar total alkaloids.

3.2 Direct sedative and hypnotic effects of total alkaloids from LEU on mice

The experiment showed that the total alkaloids in LEU could directly induce 50%–80% mice to fall asleep (Table 1). Compared to the saline-negative control group, the total alkaloids could significantly reduce the frequency of the spontaneous activity ($P < 0.01$). With the total alkaloids increasing, the sleep time of mice was significantly lengthened and the hypnotic effects were strengthened. The anatomy of the mice did not exhibit toxicity symptoms within two weeks after ig administration in the total alkaloids groups. There was no abnormality in the functions of heart, lung, spleen, liver, kidney, or gastrointestinal tract. During the experiments, the number of spontaneous activities of mice treated with total alkaloids returned to normal more quickly than that of the Diazepam-positive control group. Therefore, the total alkaloids were considered to be the more effective and safe candidate with little side effect. Further work should be needed in this field.

3.3 Effects of total alkaloids on hypnosis of pentobarbital sodium at subthreshold or superthreshold doses

The experiments of pentobarbital sodium at different threshold doses showed that the sleep rates of the mice within 30 min were 10%, 50%, 90%, and 100% at the doses of 25, 30, 35, and 40 mg/kg, respectively. According to the experimental results, the subthreshold dose of pentobarbital sodium that caused the mice to fall asleep was established as 25 mg/kg, while the superthreshold dose at which all mice in the group were asleep was 40 mg/kg.

Table 2 shows that the total alkaloids and pentobarbital sodium at subthreshold dose had excellent synergic effect. Compared to the saline-negative control group, the synergic effect could significantly increase the sleep rate, shorten the sleep latency, and lengthen the sleep time.

Table 1 Direct sedative and hypnotic effects of total alkaloids in LEU on mice ($n = 10$)

Groups	Doses / (g·kg ⁻¹)	Sedation rates / %	Sleep rates / %	Frequency of spontaneous activities (within 5 min)	Sleep time / min
total alkaloids	1.0	100**	80** $\Delta\Delta$	48 \pm 17** $\Delta\Delta$	11.71 \pm 2.29
	0.5	100**	70** $\Delta\Delta$	50 \pm 19** $\Delta\Delta$	7.95 \pm 1.91
	0.33	100**	50** $\Delta\Delta$	62 \pm 15** $\Delta\Delta$	4.54 \pm 2.05
saline-negative control	0	0	0	149 \pm 32	0
Diazepam-positive control	0.005	100	0	85 \pm 30**	0

* $P < 0.05$ ** $P < 0.01$ vs saline-negative control group, $\Delta P < 0.05$ $\Delta\Delta P < 0.01$ vs Diazepam-positive control group; same as below

Compared to the saline-negative control group, the total alkaloids could significantly shorten the sleep latency of the mice treated with pentobarbital sodium at the superthreshold dose ($P < 0.05$), especially in the high- and mid-dose groups ($P < 0.01$), and significantly lengthen the sleep time ($P < 0.01$) (Table 3). And there was no significantly statistical difference in drug efficacy between the total alkaloids and Diazepam groups.

Table 2 Effect of total alkaloids on sedation and hypnosis of pentobarbital sodium at subthreshold dose (25 mg/kg) in mice ($n = 10$)

Groups	Doses / (g·kg ⁻¹)	Sleep rates / %	Sleep latencies / min	Sleep time / min
total alkaloids	1.0	90**	7.76 ± 2.19	20.66 ± 6.71
	0.5	80*	11.14 ± 3.88	13.40 ± 3.79
	0.33	80*	13.58 ± 4.35	13.30 ± 3.77
saline-negative control	0	10	9.06	7.33
Diazepam-positive control	0.005	100	12.23 ± 6.32	19.55 ± 10.05

Table 3 Effect of total alkaloids on sedation and hypnosis of pentobarbital sodium at superthreshold dose (40 mg/kg) in mice ($n = 10$)

Groups	Doses / (g·kg ⁻¹)	Sleep rates / %	Sleep latencies / min	Sleep time / min
total alkaloids	1.0	100	3.48 ± 1.07**	71.72 ± 25.93**
	0.5	100	4.70 ± 1.97**	50.78 ± 30.72**
	0.33	100	5.38 ± 1.88*	42.32 ± 19.92**
saline-negative control	0	100	9.78 ± 4.43	15.88 ± 9.75
Diazepam-positive control	0.005	100	6.05 ± 3.13*	63.55 ± 17.85**

Table 4 Effect of total alkaloids on convulsion in mice ($n = 10$)

Groups	Doses / (g·kg ⁻¹)	Convulsion rates / %	Convulsion latencies / min
total alkaloids	1.0	60	11.37 ± 1.48**
	0.5	80	9.63 ± 0.55**
	0.33	100	5.82 ± 2.15
saline-negative control	0	100	3.65 ± 1.42
Diazepam-positive control	0.005	60	13.35 ± 4.62*

4. Discussion

The results demonstrate the favorable hypnotic and anticonvulsant activities of total alkaloids in LEU. The experiments show that the method used for extracting the total alkaloids from LEU in an acid environment is both practical and reliable. The total alkaloids not only have stronger and direct hypnotic effects, but also produce potent drug efficacy while synergized with pentobarbital sodium in preventing convulsions.

Compared to Diazepam (Xie et al, 2010), the mice treated with total alkaloids recover more quickly both from sleep state and convulsions caused by nikethamide to spontaneous activity. Meanwhile, the little side effects (dose less than 1 g/kg has no toxicity in mice) provide a basis for the further study and development of CMM with hypnotic effects. This study confirmed the hypnotic and anticonvulsant activities of the total alkaloids in LEU, indicating that the utilization of them may provide health benefits.

3.4 Effects of total alkaloids on convulsion

Table 4 showed that the total alkaloids could effectively decrease the convulsion rate caused by nikethamide (0.01 mL/g) in mice. The total alkaloids at high- and mid-dose could more significantly lengthen the convulsion latency and reduce the convulsion rate ($P < 0.01$). The drug efficacy was similar to that of Diazepam, without any significant difference.

According to the latest issues, the insomnia treatment emphasizes not only on medicinal treatment but also on the reasonable use of calm hypnosis drugs with improving the symptoms of patients rapidly. Thus the confidence of the treatment for patients was built up. The hypnotic effects would be more receptor selective with shorter half-life and less adverse reaction gradually, and develop from barbiturates to benzodiazepine with more safety (Wang et al, 2009). A common advantage of CMM is that there is little adverse side effect and slightly dose-dependent. Total alkaloids obtained from *E. ulmoides* should be the excellent candidate for the development as a hypnotic drug. However, the monomer compound of the active ingredients has not been separated and purified, and the mechanism of the hypnotic effects has not been clarified. Further study is still required.

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References

- Calisto V, Esteves VI, 2009. Psychiatric pharmaceuticals in the environment. *Chemosphere* 77(10): 1257-1274.
- Cheng GL, 2006. Study of the active component of *Eucommia* and its mechanism. *Chin Patent Drug* 28: 723-725.
- Dou W, Zhao ZX, 2004. Research progress of sedative-hypnotic tolerability. *J Neurosci* 37: 459-462.
- Frank GB, Jhamadnas K, 1970. Effects of general stimulant drugs on

- the electrical responses of isolated slabs of cat's cerebral cortex. *Br J Pharmacol* 39: 716-723.
- Fu JW, Qiao W, Chen ZH, 2005. Study on sedative and hypnotic effects of the total alkaloids of wild jujube kernel. *J Tianjin Med Univ* 11: 52-54.
- Geng JZ, Xu FX, 2007. Progress of research on the alkaloids physiology and its extraction and separation. *Summarize and Comment* 10: 1-4.
- Guan SY, Su WW, 2003. Research progress of *Eucommia*'s chemistry component and its pharmacology. *Tradit Chin Med Mater* 26: 126-129.
- Li YF, Su YM, Li TL, Yan XK, 2012. Material basis of Sini Powder with sedative-hypnotic effect. *Chin Tradit Herb Drugs* 43(7): 1361-1365.
- Li LK, Xia JJ, Shi RF, Wang CH, 2007. Application of extraction of natural alkaloids and resin method in its purification. *Ion Exch Absorpt* 23: 475-480.
- Lian XM, Zhu WX, Bai XT, 2007. Determination of active ingredients of male *Eucommia* flowers and the mechanism of hypnosis. *Food Sci and Tech* 3: 203-205.
- Liu N, Liu CS, 2002. Research progress of *Eucommia* in China and abroad. *Med J Natl Def Force Southwest China* 12: 450-451.
- Michael JP, 2007. Quinoline, quinazoline and acridone alkaloids. *Nat Prod Rep* 14: 605-618.
- Talaty N, Takáts Z, Cooks RG, 2005. Rapid *in situ* detection of alkaloids in plant tissue under ambient conditions using desorption electrospray ionization. *Analyst* 130: 1624-1633.
- Wang GZ, Hu HT, Gao J, Zhao Q and Zhang TL, 2009. The research progress of sedative hypnotic drugs. *China Foreign Med Treat* 28: 101-103.
- Wang J, Chen J, 2012. Sedation and hypnotism of Sugar-free Ningxin Anshen Syrup in mice. *Drug Clin* 27(4): 360-362.
- Xie Y, Tang YY, Wei JJ, 2010. The effect of diazepam on the weight, pain threshold and independent activity of anxious mice and depressive mice. *Chin Pharmacol Bull* 26:1529-1530
- Yu CY, Wang XL, 2006. Application progress of alkaloids extraction and purification technique. *Chem Ind Eng Prog* 25: 259-264.
- Zhan YC, Zhang YM, Guo DY, 2001. Research on the anti- hypertensive effect of *Eucommia* leaves. *Chin Patent Drug* 23: 418-420.
- Zhang GL, Miao MS, Shia JJ, Yang YL, 2007. Synergistic action on hypnosis Yinao capsules with pentobarbital sodium of threshold and sub-threshold dosages. *Neural Regeneration Res* 2: 91-94.
- Zhao JT, Zhang C, 2003. Research and application of different parts of *Eucommia*. *J Practical Med Tech* 10: 1025-1027.