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Sedative, hypnotic and anticonvulsant activities of the ethanol fraction from *Rhizoma Pinelliae* Praeparatum

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ABSTRACT

Ethnopharmacological relevance: Rhizoma Pinelliae Praeparatum is the product of raw *Rhizoma Pinellia* processed with alkaline solution and Licorice, which had been widely used for treatment of insomnia in traditional Chinese medicine. The present study aimed to investigate the sedative, hypnotic and anticonvulsant activities of ethanol fraction from *Rhizoma Pinelliae* Praeparatum (EFRP) and to determine whether these effects were related to GABAergic mechanism.

Materials and methods: The sedative, hypnotic and anticonvulsant activities of EFRP were investigated with locomotion activity, pentobarbital-induced sleeping and nikethamide (NKTM)-induced convulsion tests, respectively. Additionally, the effects of flumazenil (an antagonist of $GABA_A$ receptor) and L-malic acid (blocker of synthetic enzyme for GABA) on the hypnotic activity of EFRP were evaluated.

Results: EFRP at dose of 12 g/kg significantly inhibited the locomotion activity of mice. EFRP showed synergic effect on pentobarbital-induced sleeping by increased numbers of mice falling asleep, reduced the sleep latency and prolonged the sleeping time. L-malic acid and flumazenil inhibited the augment effects of EFRP on pentobarbital-induced sleeping. EFRP promoted a significant protection to NKTM-induced convulsion, by prolonged the death latency and decreased mortality.

Conclusion: EFRP possessed sedative, hypnotic and anticonvulsant activities and these activities may be related to the GABAergic system.

1. Introduction

Numerous herbal medicines have been used for the treatment of central nervous system (CNS) disease, especially for chronic conditions such as anxiety, depression, insomnia, headaches and epilepsy, which do not respond well to conventional treatments (Phillipson, 2001; Carlini, 2003). *Rhizoma Pinelliae*, the tuber of *Pinelliae ternata* (Thunb.) Breit. (Araceae), has been widely used in clinic for antiemetic, antitussive, sedative and anti-inflammatory purposes (Marki et al., 1987). *Pinelliae ternata* is a traditional Chinese medicine herb which is distributed in China, Korea and Japan. In China, *Pinelliae ternata* has been used for the treatment of cough, vomiting, infection and inflammation since ancient times.

Raw *Pinellia ternata* could cause irritant toxicity to oral, throat and gastrointestinal mucosa, vomit and diarrhea. So it was always used in its preparation forms in clinic, especially *Rhizoma Pinellia* Praeparatum. As recorded in Chinese Pharmacopoeia (2005 and 2010 Edition), *Rhizoma Pinelliae* Praeparatum is the product of raw *Pinellia* processed with alkaline solution and Licorice. *Rhizoma Pinelliae* Praeparatum is the component of many decoctions in traditional Chinese medicine, such as Xiaochaihu-Tang (Japanese name Sho-seiryu-to) which is used clinically for the therapy of allergic rhinitis, bronchitis, bronchial asthma and cold symptoms (Nagai et al., 2004). Modern pharmacological studies showed that *Rhizoma Pinelliae* Praeparatum possessed multiple activities, such as antitussive, expectorant, antiemetic, antitumor, antibacterial, anti-inflammation, antioxidant and sedative-hypnotic (Wang et al., 2008), etc. Phytochemical studies of *Pinelliae or Rhizoma Pinelliae* Praeparatum have been extensively reported including alkaloids (Zhao et al., 1990), essential oils (Wang et al., 1995), polysaccharides (Gonda et al., 1994; Tomoda et al., 1994), amino acids (Li et al., 1990), organic acids (Zhang et al., 2002) and proteins (Tao et al., 1981).

The present study was focused on the CNS effects of *Rhizoma Pinelliae* Praeparatum. As a traditional Chinese medicine, *Pinelliae* had been used for treatment of insomnia in combinative forms for about two thousand years, such as Banxia–Xiexin-Decoction, Banxia–Shumi-Decoction, Banxia–Kucao-Decoction (Tao, 2001) and so on. Several literatures had reported about the sedative, hypnotic and anticonvulsant activities of *Pinelliae* in combination

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forms. Combination of *Pinelliae* total alkaloids and *Uncarial* total alkaloids demonstrated synergistic effects in anticonvulsant action; the mechanism might be related to decreasing the excitability of glutamatergic neurons and increasing the inhibition of GABAergic neurons (Cheng et al., 2007). Combination of *Pinelliae* ethanol extract and sophora aqueous extract significantly prolonged sleeping time of mice induced by sodium pentobarbital (Zhan, 2008). The effects of *Pinellia ternata* (*Thunb*) Breit *Pinellia pedatisecta* Schott and *Typhonium Flagelliforme* Blume on pentobarbital induced sleep were compared, which indicated the ethanol extract of *Pinellia ternata* possessed potentiating effect on sodium pentobarbital-induced hypnosis (Zhan et al., 2006). However, to the best of our knowledge, there was no study reported about the active fraction and its sedative, hypnotic and anticonvulsant activities systematically. Therefore, the present work was to investigate the effects of ethanol fraction of *Rhizoma Pinelliae* Praeparatum on locomotion activity, pentobarbital-induced sleeping, and NKTM-induced convulsions in mice.

2. Materials and methods

2.1. Animals

Male ICR mice (18–22 g) were purchased from Animal Center of Jiangsu University, and housed under controlled environment ($22 \pm 2\,^{\circ}C$, 12 h light/dark cycle, free access to food and water). The mice were fasted for 12 h before the experimental procedures. All experiments were carried out between 8:00 and 13:00 in a quiet room with temperature 22–24 °C.

All procedures were conducted in accordance with experimental animal institutional policies, and approved by the Jiangsu University Committee on Animal Care and Use.

2.2. Plant material and ethanol extraction

Raw *Rhizoma Pinelliae* and *Rhizoma Pinelliae* Praeparatum (Origin of Nanchong, Sichuan Province, China), produced by Bozhou Yuan'guang Chinese Medicine Co., Ltd., and purchased from Chi Lin Dispensary of Jiangsu Province in China, which were authenticated by Li Chen, Chief Pharmacist of Zhenjiang Food and Drug Administration. The voucher specimen of Raw *Rhizoma Pinelliae* and *Rhizoma Pinelliae Praeparatum* was deposited at the School of Pharmacy, Jiangsu University with a number 20090627.

Rhizoma Pinelliae Praeparatum was crushed into crude powders and extracted thrice with 60% ethanol at 85 °C for 2 h. The extract was filtered and concentrated with a rotary vacuum evaporator followed by lyophilization. The dried extract was abbreviated as EFRP. The yield of EFRP was 2.78% (w/w).

2.3. Drugs

EFRP and diazepam pills (DZP, Jiangsu Jichuan Medical Co. Ltd., China) were suspended with distilled water. Pentobarbital (Serve, Shanghai Chemical Reagent Corporation, China) and L-malic acid were dissolved and nikethamide injection (NKTM, 0.375 g/1.5 ml, Tianjin Pharmaceutical Group, Tianjin, China) was diluted with physiological saline. Flumazenil (Sigma) was dissolved in 1% DMSO/water.

2.4. Locomotion activities in mice

The locomotion activity of mice was measured with a YLS-1A Multi-autonomous Activity Instrument (Shandong Academy of Medical Sciences, Jinan, China), which was consisted of a micro-computer control system and five activity cages (12 cm × 10 cm × 12 cm, length × width × height). The micro-computer control system detects thermal infrared emitted by small animals and counts locomotion activity by recording the locomotion originated from crossing movements (walking, turn round) and/or longitudinal movements (lifting forelimb, climbing), but eliminates disturbances of tinny movements such as preening, trembling, tail-flicking and so on.

EFRP and DZP were administered orally (0.2 ml/10 g, volume/body weight) 30 min before the test. Animals were placed individually in the activity cages 5 min before the test to adapt. The numbers of locomotion activity was counted for 5 min.

2.5. Pentobarbital-induced sleeping

Observers were blind to the drug treatment. EFRP and DZP (0.2 ml/10 g, volume/body weight) were administered intragastric (i.g.) 30 min before the pentobarbital intraperitoneally (i.p.) injection.

The present study was to use 45 mg/kg (i.p., 0.1 ml/10 g, volume/body weight) pentobarbital as hypnotic dosage (sleep onset 100%) and 25 mg/kg (i.p., 0.1 ml/10 g, volume/body weight) as sub-hypnotic dosage (sleep onset 0%). Following pentobarbital administration, each mouse was observed for the sleep onset, a mouse losing righting reflex over 3 min was considered to be asleep. The time elapsed between pentobarbital injection and loss of righting reflex was recorded as sleep latency, and that elapsed between loss and recovery of righting reflex was recorded as sleeping time. In the sub-hypnotic pentobarbital test, sleeping time of animals failed to fall asleep within 15 min after pentobarbital injection was noted as 0 min, and sleep latency was noted as 15 min.

To investigate the possible mechanism involved in the hypnotic activity of EFRP, mice were pretreated with flumazenil (3.5 mg/kg), an antagonist of $GABA_A$–benzodiazepine receptor or L-malic acid (600 mg/kg), blocker of synthetic enzyme for GABA.

2.6. Nikethamide-induced convulsions

In this test, NKTM was administered intraperitoneally (400 mg/kg, i.p., 0.1 ml/10 g, volume/body weight) 30 min after EFRP administration. Following the NKTM injection, animals were observed (60 min) for occurrence of seizure and presence of clonic convulsion. The duration between administration of NKTM and onset of convulsion was recorded as convulsion latency and that between administration of NKTM and death as death latency. Animals devoid of seizure were considered protected.

2.7. Statistical analysis

All data were presented as mean ± SEM. For statistical comparison, results were analyzed by One-way analysis of variance (ANONA) Students–Newman–Keuls test (SNK). For the sub-hypnotic dosage of pentobarbital test, a Chi-square test was used to compare the number of mice fall asleep. $p < 0.05$ was considered statistically significant difference.

3. Results

3.1. Effect of EFRP on the locomotion activity in mice

The sedative activity of EFRP was investigated by the locomotion test of mice. EFRP (8 and 12 g plant material/kg weight) reduced the locomotion activity of mice dose dependently from 184.0 ± 14.20 (distilled water) to 149.0 ± 32.8 ($p > 0.05$) and 103.6 ± 22.5 ($p < 0.05$), respectively (Table 1). Positive control DZP (4 mg/kg) also significantly decreased the locomotion activity of mice ($p < 0.01$).