Evaluation of the sedative and hypnotic effects of ethanol-extract isolated from *Herba Agrimoniae* in mice

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Abstract. The purpose of this study was to evaluate the sedative and hypnotic activities of ethanol-extract isolated from *Herba Agrimoniae* on the central nervous system. *Herba Agrimoniae* has been used as a traditional herbal medicine for treating various cancers and diseases in traditional Chinese medicine. In the experiments, the extract of *Herba Agrimoniae* showed significant effects on mice, including reduced spontaneous activity, increased sleep ratio, shortened sleep latency and lengthened sleep time with a subthreshold or superthreshold dose of pentobarbital sodium. These findings confirmed that ethanol-extract isolated from *Herba Agrimoniae* had sedative and hypnotic effects and has potential to be preferable for the treatment of insomnia.

Keywords: Insomnia, *Herba Agrimoniae*, Sedative–hypnotic effects, Pentobarbital sleep

Introduction

Insomnia defined as persistent difficulty in falling or staying asleep that affects daytime function, can induce significant psychological and physical disorder. Most patients engage in long-term use of benzodiazepines analogs to treat insomnia[1-3]. However, these drugs have limited benefits with significant side-effects, such as impaired cognitive function, memory and paradoxical effects such as aggression or behavioral disinhibition occasionally occur[4, 5]. In addition, long-term use is controversial because of concerns about adverse psychological and physical effects, decreasing effectiveness, and physical dependence and withdrawal[6, 7]. The search for novel herbal constituents from medicinal plants may provide better candidates for pharmacotherapeutics.

Herba Agrimoniae is a well known traditional medicinal plant with diverse properties including anti-oxidant, anti-inflammatory, analgesic and anti-tumor activity[8-10]. The pharmacological activity of *Herba Agrimoniae* may primarily attributed to phenolic compounds such as agrimonin, catechin, quercetin and rutin[11, 12]. The research on *Herba Agrimoniae* has focused mainly on the prevent or treat various diseases such as abdominal pain, sore throat, headaches, bloody discharge, parasitic infections and eczema [9, 13, 14]. Many studies have indicated that *Herba Agrimoniae* ethanol-extract can decrease blood glucose for alloxan-induced diabetic mice, and also showed that significant hypoglycemic effect for diabetic model mice whom caused by glucose, adrenaline and streptozotocin[15]. However, the effects of ethanol-extract isolated from *Herba Agrimoniae* on the central nervous system has not been revealed. Therefore, the present work was to investigate the effects of ethanol isolated of *Herba Agrimoniae* on locomotion activity, pentobarbital-induced sleeping in mice.

Materials and methods

Animals

Adult male Kunming mice (weighing 25 ± 2 g) were provided by the Animal Test Center of China Three Gorges University, license number: SCXK (E) 2017-0012. They were housed under controlled environment ($22 \pm 2^\circ\text{C}$, 12h light/dark cycle, free access to food and water). After 7 days of acclimatization, all mice were randomly divided into different groups ($n=12$). This study was approved by the Jiangnan university experimental animal ethical review committee (license No 201705002, date: 9/30/2017), and the experimental procedures are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication no. 85-23, revised 1985).

Drugs and materials

Herba Agrimoniae were collected from a Chinese medicine shop of Wuhan in China according to the guidelines of the Chinese Pharmacopoeia (2010). Pentobarbital sodium was obtained from JingDu biological technology Co. Ltd. (Shanghai, China), diazepam (DZP) injection was provided by Jiangnan University Hospital. All other chemicals, reagents and equipment were of the highest grade available.

Isolation of Agrimony

Take Agrimony 300 g, set 5000 mL round bottom flask, add 90% ethanol 3000 mL reflux extraction 2 h, suction filtration, the residue plus 90% ethanol 2000 mL reflux extraction 2 times each 1 h. The filtrate was filtered, the filtrate was combined, ethanol was recovered, concentrated to a concentration of 1 g crude drug/mL, add an equal volume of pure water washed a total of three times to ethanol-free, dried in a vacuum oven and suspended at 400 mL water extract Herba Agrimoniae which be alternated. The dried extract was abbreviated as EIHA.

Treatments

For intraperitoneal (i.p.) injection, sodium pentobarbital was dissolved in physiological saline. The present study used 60 mg/kg (i.p.) as the hypnotic dose of sodium pentobarbital (with a 100% rate of sleep onset) and 45 mg/kg (i.p.) as the subhypnotic dose (with a 0-10 % rate of sleep onset). EIHA (3, 6 and 12 g/kg) and DZP (2 mg/kg) were dissolved with 1% dimethyl sulfoxide (DMSO) and administered intragastric (i.g.) 30 min before sodium pentobarbital administration (i.p.).

Inner open-field behavior test

The sedative activity was investigated by determining the spontaneous locomotor activity of mice in an open field. Animals were placed in an open field experimental video analysis system, and acclimated to the activity cages individually for 5 min. EIHA and DZP were administered orally (0.1 mL/10 g, volume/body weight) 30 min before the test. The numbers of locomotion activity was counted for 5 min.

Pentobarbital-induced sleep test

EIHA and DZP were administered intragastric (i.g.) 30 min before the pentobarbital (i.p.) injection. Following sodium pentobarbital injection, each mouse was observed for the onset of sleep. When the mice lost the righting reflex for over 1 min, they were considered to be asleep. The loss of righting reflex was defined as a failure of the mouse to right itself for at least 10 s after being placed on its back. Time elapsed between the administration of sodium pentobarbital and sleep latency was recorded as the time of pentobarbital injection to the time of sleep onset, and sleep duration was defined as the elapsed time between the righting reflex loss and recovery. In the subhypnotic dosage of sodium pentobarbital treatment test, the percentage of sleep onset was calculated as follows: $\text{sleep onset (\%)} = \frac{\text{no. falling asleep}}{\text{total no.}} \times 100$.

Statistical analysis

All data are presented as the mean $\pm$ S.E.M. For statistical comparisons, the results were analyzed by a one-way analysis of variance (ANOVA) followed by the Students–Newman–Keuls test (SNK) for post-hoc comparisons. For the subhypnotic dosage of pentobarbital test, a chi-square test was used to compare the number of mice that fell asleep. Differences with $P < 0.05$ were considered statistically significant.

Results

Effects on spontaneous activity of mice

The sedative activity of EIHA was investigated by the locomotion test of mice. EIHA (12 g/kg) reduced the locomotion activity of mice dose from 231.00 ± 7.61 to 195.06 ± 6.67 ($P < 0.05$) (Fig. 1). Positive control DZP (2 mg/kg) also significantly decreased the locomotion activity of mice ($P < 0.01$).

Fig. 1. Inhibitory effect of EIHA on spontaneous locomotor activity in mice. Five minutes after administration of DZP (2 mg/kg, i.g.) and EIHA (3, 6 and 12 g/kg, i.g.), spontaneous locomotor activity is measured. Each column represents the mean $\pm$ S.E.M (n=12). * $P < 0.05$ compared with the vehicle group.

Influence on the sedative effect with subthreshold dose of pentobarbital sodium

As shown in Table 2, EIHA (12 g/kg, i.g.) pretreatment significantly increased the rate of sleep onset with a subhypnotic dose of pentobarbital (40 mg/kg, i.p.) compared with that of the vehicle group.

Table 2. The effect of EIHA on the sleep onset of mice treated with a subhypnotic dose of sodium pentobarbital.

Group	Dosage	No. of falling asleep/total	Sleep onset (%)
Vehicle	—	12/12	0
DZP	2 mg/kg	11/12	91.7*
EIHA	3 g/kg	3/12	25
	6 g/kg	4/12	33.3
	12 g/kg	8/12	66.7*

Thirty minutes after administration of DMSO, DZP, and EIHA, sodium pentobarbital (40 mg/kg, i.p.) was given to mice. The number of mice falling asleep was recorded (n=12). * $P < 0.01$, compared with the vehicle group, chi-square test.

Influence on the sedative effect with superthreshold dose of pentobarbital sodium

In mice treated with a hypnotic dose of pentobarbital (60 mg/kg, i.p.), EIHA significantly potentiated the hypnotic effects of pentobarbital by reducing sleep latency at the dosages of 12 g/kg ($P<0.05$) (Fig. 3A) and prolonged sleep duration at the dosages of 12 g/kg ($P<0.05$) (Fig. 3B).

Fig. 3. Effect of EIHA on the hypnotic response to pentobarbital-induced sleep in mice. The sleep latency (A) and the sleep duration (B) are assessed. All data are presented as mean $\pm$ S.E.M (n=12), * $P<0.05$ compared with the vehicle group.

Conclusion

In conclusion, this work represents the EIHA had sedative, hypnotic actions. But the mechanism of regulating sleep of EIHA is not clear. Further investigation will be necessary for active compounds and extensional mechanism.

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