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Ferulic acid potentiates pentobarbital-induced sleep via the serotonergic system

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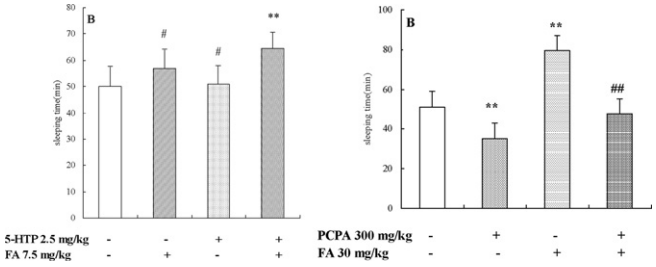
HIGHLIGHTS

- We investigated the sedative and hypnotic activities of ferulic acid.
- Ferulic acid inhibited the locomotion activity of mice.
- Ferulic acid potentiated the hypnotic effect of pentobarbital.
- Ferulic acid exerted sedative and hypnotic activity via the serotonergic system.
- Ferulic acid may be a pleasant candidate in therapy of insomnia.

GRAPHICAL ABSTRACT

Ferulic acid (4-hydroxy-3-methoxycinnamic acid, FA) is a widespread natural phenolic compound which exert some bioactivity. The sedative and hypnotic effects of FA and possible mechanisms were investigated through behavioral pharmacology methods. The extract demonstrated that FA possessed sedative and hypnotic activities, which may mediated by serotonergic system.



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ABSTRACT

Ferulic acid (4-hydroxy-3-methoxycinnamic acid, FA) is a widely distributed natural phenolic compound that is abundant in many plant tissues and foods. This study investigated possible mechanisms underlying the sedative–hypnotic effect of FA through behavioral pharmacology methods. FA showed dose-dependent sedative effects on locomotion activity in normal mice. FA also significantly potentiated pentobarbital-induced (45 mg/kg, i.p.) sleep by prolonging sleeping time and shortening sleep latency in a dose-dependent manner. These effects were augmented by the administration of 5-hydroxytryptophan (5-HTP), a precursor of 5-hydroxytryptamine (5-HT). With a sub-hypnotic dose of pentobarbital (25 mg/kg, i.p.), FA significantly increased the rate of sleep onset and exhibited a synergistic effect with 5-HTP (2.5 mg/kg, i.p.). Pretreatment with *p*-chlorophenylalanine (PCPA, an inhibitor of tryptophan hydroxylase) significantly decreased the duration of pentobarbital-induced sleep, whereas FA significantly reversed this effect. These results suggest that FA has sedative–hypnotic activity, possibly mediated by the serotonergic system.

Insomnia, defined as persistent difficulty in initiating or maintaining sleep that affects daytime function, can induce significant psychological and physical disorders [10]. More than 27% of people worldwide experience insomnia, and approximately 3–10% of people are chronic and frequent users of hypnotics [7]. Clinically, benzodiazepines are the most widely used hypnotic agents prescribed for insomnia. However, benzodiazepines have many unpleasant side effects, including drug dependence, drug tolerance, rebound insomnia, and amnesia. New types of hypnotics, such as zolpidem and zolpiclone, also show some side effects [2,9].

Ferulic acid (4-hydroxy-3-methoxycinnamic acid, FA) is a ubiquitous natural phenolic acid found in many plant tissues and foods, including *Ferula assafoetida* L., *Ligusticum chuanxiong* Hort, *Cimicifuga foetida* L., cabbages, wheat, rice bran, tomatoes, and onions. FA exhibits many bioactivities including antioxidant, antimicrobial, radio-protective, hypertensive, and anti-carcinogenic properties

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[1,11,16]. Currently, FA is widely used in the medical, food, and cosmetic industries. Because FA has low toxicity and is metabolized quickly, it has been approved as a food additive in some countries.

FA can pass through the blood-brain barrier and reach the brain; its pharmacokinetics complies with the open one-compartment model in vivo. It can be absorbed completely and rapidly, metabolized quickly, and excreted by the kidneys without apparent accumulation in vivo [4]. FA exerts neuro-protective effects against oxidative stress-related apoptosis after cerebral ischemia and significantly reduces cerebral infarct in a transient middle cerebral artery occlusion (MCAO) model [5,6,15]. Additional studies have reported that FA can attenuate neuronal cell death caused by the uptake of oxidized low-density lipoprotein, hydroxyl and peroxyl radicals in vitro [12,19]. FA may also attenuate neuronal cell death during MCAO involved in inhibition of Akt signaling pathway inactivation and maintenance of the interaction between phosphor-Bad and 14-3-3 [15]. FA may also have sedative activity. However, to the best of our knowledge, no previous studies have reported any hypnotic-sedative activity of FA. The present study investigated the hypnotic effects and possible mechanisms of FA on pentobarbital-induced sleep in rodents.

A total of 390 male ICR mice (weight 18–22 g, Grade I; purchased from Tianjin Medical University) were used in this study. The mice were housed under controlled environmental conditions (temperature 22 ± 2 °C, humidity 50 ± 10%, 12 h light/dark cycle and light on at 0600) and given ad libitum access to food and water. The mice were acclimated 1 week before testing. The mice were fasted for 12 h prior to the onset of the experiments. The experiments were carried out between 0800 and 1300 in a quiet room with temperatures ranging from 22 to 24 °C. All procedures were conducted in accordance with the European Community guidelines for the use of experimental animals and approved by the Tianjin Medical University Committee on Animal Care and Use.

For intragastric (i.g.) administration (0.2 ml/10 g, volume/body weight), FA (Sigma–Aldrich, St. Louis) and L-malic acid (L-MA) were dissolved and diazepam injection (DZP, 10 mg/2 ml, manufactured by People's Pharmaceutical Manufacturer, Tianjin, China) was diluted with 0.5% dimethyl sulfoxide (DMSO). For intraperitoneal (i.p.) injection (0.1 ml/10 g, volume/body weight), 5-HTP (Alfa Aesar China Ltd., Beijing, China) and pentobarbital (Serve, Shanghai Chemical Reagent Corporation, China) were dissolved with physiological saline. For subcutaneous (s.c.) injection (0.1 ml/10 g, volume/body weight), PCPA (Sigma–Aldrich, St. Louis) was suspended in 0.5% gum acacia/physiological saline.

The locomotion activity of mice was measured using an YLS-1A Multi-autonomous Activity Instrument with five activity cages (Shandong Academy of Medical Sciences, Jinan, China) [23]. FA and DZP were administered orally (0.2 ml/10 g body weight). Twenty-five minutes after injection, mice were acclimated to the activity cages individually for 5 min; then, the locomotion activity of each mouse was measured for 5 min.

The present study used 45 mg/kg as the hypnotic dosage of pentobarbital (sleep onset 100%) and 25 mg/kg as sub-hypnotic dosage (sleep onset 0%). FA and DZP were administered (i.g.) 45 min prior to pentobarbital administration (i.p.). 5-HTP was injected (i.p.) 15 min prior to pentobarbital administration (i.p.). PCPA-pretreated mice received an injection of PCPA (300 mg/kg, s.c.) between 0800 and 0900, 24 h prior to the injection of pentobarbital. L-MA pretreated mice received an injection of L-MA (600 mg/kg) for 5 days between 0800 and 0900. On the fifth day, mice received L-MA 60 min prior to pentobarbital injection.

Observers were blind to the drug treatment. 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 the loss of righting reflex was recorded as the sleep latency. The time elapsed between the loss and recovery of the righting reflex was recorded as sleeping time.

Table 1
Effect of FA on locomotion activity in mice ($n = 10$).

Groups	Locomotion activity (times/5 min)	Inhibitory (%)
Vehicle	165.4 ± 12.7	0
DZ 2.0 mg/kg	12.7 ± 6.8	92.3**
FA 7.5 mg/kg	153.2 ± 16.3	7.4
FA 15.0 mg/kg	121.7 ± 17.6	26.4*
FA 30.0 mg/kg	109.8 ± 15.5	33.6**

* $P < 0.05$, compared with vehicle, ANOVA/SNK-test.
** $P < 0.01$, compared with vehicle, ANOVA/SNK-test.

All data are presented as the mean ± 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 sub-hypnotic dosage of pentobarbital test, a chi-square test was used to compare the number of mice that fell asleep. $P < 0.05$ was considered statistically significant difference.

Thirty minutes after FA administration, the locomotion activity of the mice was measured for 5 min. FA reduced the locomotion activity in a dose-dependent manner from 165.48 ± 12.7 (vehicle) to 153.2 ± 16.3 (7.5 mg/kg, $P > 0.05$), 121.7 ± 17.6 (15.0 mg/kg, $P < 0.05$), and 109.8 ± 15.5 (30.0 mg/kg, $P < 0.01$) (Table 1). The positive control DZP (2 mg/kg) also significantly decreased the locomotion activity ($P < 0.01$).

FA showed synergistic effects with pentobarbital. In mice treated with the hypnotic dosage of pentobarbital (45 mg/kg), FA significantly shortened the sleep latency (Fig. 1A) and prolonged the sleeping time (Fig. 1B) in a dose-dependent manner at 15 mg/kg ($P < 0.05$) and 30 mg/kg ($P < 0.01$). In mice treated with the sub-hypnotic dosage of pentobarbital (25 mg/kg), FA significantly increased the rate of sleep onset in a dose-dependent manner

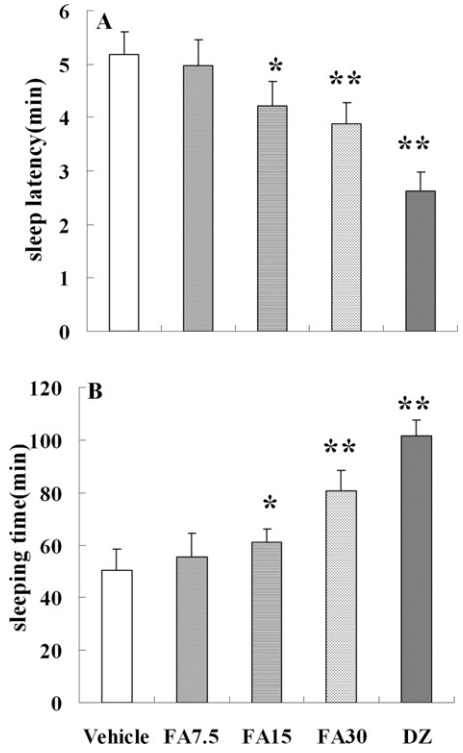

Fig. 1. Effect of FA on the hypnotic response to pentobarbital induced sleep in mice. The sleep latency (A) and the sleep time (B) were assessed. All data were presented as mean ± S.E.M. ($n = 10$ for vehicle; $n = 15$ for the other groups). *$P < 0.05$ and **$P < 0.01$ vs. vehicle (Student–Newman–Keuls test).