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The effects of angelica essential oil in social interaction and hole-board tests

Li Min [1], Si Wei Chen *, Wei Jing Li, Rui Wang, Yu Lei Li, Wen Juan Wang, Xiao Juan Mi

Department of Pharmacology, Shenyang Pharmaceutical University, Box 41, 103 Wenhua Road, 110016 Shenyang, PR China

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Abstract

In our previous studies, we have demonstrated the anxiolytic effects of angelica essential oil in three anxiety models using mice. This study aimed to characterize the similar behavior effects of angelica essential oil in the social interaction test of anxiety and the hole-board test of exploration and locomotor activity in rats. These results indicate that angelica essential oil possessed a wide range of anxiolytic properties. In the social interaction test, angelica essential oil decreased aggressive behaviors at the doses of 21 and 42 mg/kg, while the doses of 21 and 42 mg/kg significantly increased social interaction time of the high light, unfamiliar test condition and 21 mg/kg could also prolong social interaction time of the high light, familiar test condition. In the hole-board test, angelica essential oil at 10.5 mg/kg significantly increased head-dipping counts and duration. Thus, our findings suggest the potential usefulness of angelica essential oil against various types of anxiety-related disorders and social failure.

Keywords: Angelica essential oil; Anxiolytic; Social interaction; Hole-board; Rat

1. Introduction

Angelica sinensis Diels is one of the most important medicinal herbs in traditional Chinese medicine. It possesses hemogenic, analgesic activities and sedative effect, and finds application in the treatment of a range of conditions including menstrual disturbance and anemia. The volatile oil is the major pharmacological component of this herb, and ligustilide is the major chemical constituent of the volatile oil (Wang et al., 1998). In our previous studies, we have proved that angelica essential oil (AEO) has an anxiolytic effect in the elevated plus-maze, the light/dark box and the stress-induced hyperthermia paradigms in male Swiss mice (Chen et al., 2004). Although various experimental models of anxiety have been proposed to measure different types or states of anxiety, there is some uncertainty as to whether anxiety mechanisms and anxiolytic drugs are uniformly active within and between animal models (Handley and McBlane, 1993). Therefore, we are particularly interested to see whether it will exhibit similar effects in the same kind of models in rats. The paradigms we selected here are two famous tests of anxiety: the social interaction and the hole-board tests. The social interaction test is an ethologically based test that is sensitive to both anxiolytic and anxiogenic effects. It measures the duration of social interaction between two rodents meeting for the first time and is thought to be a model of social anxiety in humans (File, 1980, 1985). The hole-board has gained popularity as a model of anxiety, offering "a simple method for measuring the response of an animal to an unfamiliar environment, with advantages that several behaviors can be readily observed and quantified in this test" (Takeda et al., 1998).

* Corresponding author. Tel.: +86 24 23909448; fax: +86 24 24513031.
E-mail address: c100sw@yahoo.com.cn (S.W. Chen).
[1] Present address: Department of Phytochemistry, College of Pharmacy, Second Military Medical University of PLA, 800 Xiangyin Road, 200433 Shanghai, PR China.

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2. Methods

2.1. Animal

Male Wistar rats weighing 200–250 g (Experimental Animal Center of Shenyang Pharmaceutical University) were used. For the social interaction test, rats had been singly housed for 10 days before the experiment, and were allocated to pairs on the basis of weight. For the hole-board test, rats were housed in groups of 10. Animals were kept in a room with a controlled temperature (22±2 °C), relative humidity (55±10 °C) and illumination from 07:00 to 19:00 and they had free access to food and water. All experiments were carried out between 10:00 and 16:00.

All animal treatments were strictly in accordance with the National Institutes of Health Guide of the Care and Use of Laboratory Animals. The experiments were carried out under the approval of the Committee of Experimental Animal Administration of the University.

2.2. Drugs

Angelica essential oil (supercritical CO_2 extract, containing 75.0% ligustilide analyzed by GC-MS) was purchased from Kunming Biochemistry and Fragrance Co. Ltd (Kunming, China). Diazepam was obtained from Hubei Pharmaceutical Factory (Hubei, China). Tween 80 was purchased from Shenyang Dongxing Reagent Factory (Shenyang, China). Diazepam and angelica essential oil were both dissolved in 3% concentration of Tween 80. Control animals received 3% Tween solution only. The doses of angelica essential oil tested here were based on our preliminary experiments using mice.

2.3. Procedures

2.3.1. Social interaction test

The test procedure for social interaction test was similar to that described by File and Pellow (1985). The test arena was a black Plexiglas box, 60×60×35 cm, with the base divided into 9 cm squares by lines of white tape. The light intensity of the arena floor was 380 lx. Two test conditions were performed: high light, unfamiliar arena (HU) and high light, familiar arena (HF). On day 1 of testing, each rat was randomly assigned according to body weight (<15 g difference) to an unfamiliar partner in groups of 12 animals (six pairs) which were subsequently administered the appropriate drug. These rats were then replaced into their home cage until testing. Following appropriate pretreatment time, members of each pair of unfamiliar rats were placed in opposite corners of the arena and observed for social interaction behaviors and overall locomotor activity for 10 min. At the end of this period the rats and any faecal boluses were removed and the arena wiped with a damp cloth. Social interaction time (in s) per pair of rats was measured as time of sniffing and mutual grooming, adjacent lying, climbing over and crawling under the partner, approximation and following (File, 1980). Aggressive-type behaviors (e.g. kicking, aggressive grooming, biting, boxing and jumping on; see Guy and Gardner, 1985) were also scored. These were treated as separate entities since such behaviors are modulated by different pharmacological agents than social behaviors (Miczek and Winslow, 1987). Locomotor activity was measured by counting the number of squares crossed. Following completion of the first test, rats were returned to their home cages. On days 2 and 3, the rats were placed individually, undrugged, in the

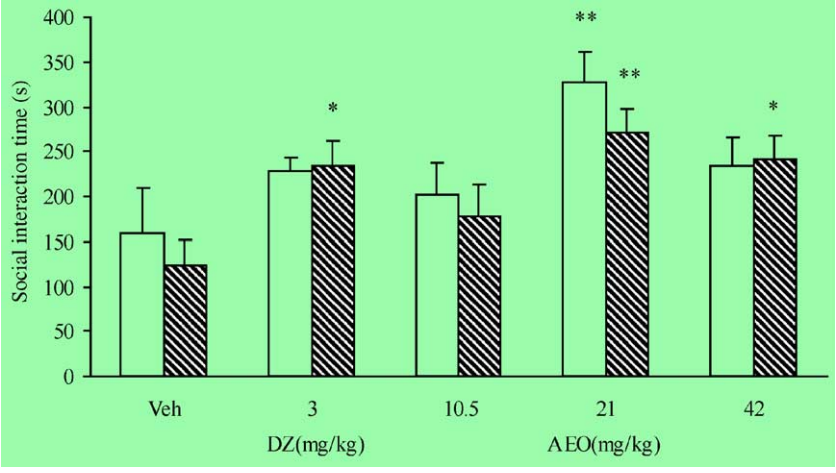

Fig. 1. Mean time±S.E.M. (s) spent in active social interaction for pairs of male rats given a 10-min trial, 40 min after drug administration. $n=6$ pairs per group. Test conditions were high light, familiar (hollowed columns) and high light, unfamiliar (hatched columns). Significance of difference: $^{*}P<0.05$, $^{**}P<0.01$ compared with vehicle condition. Bonferroni planned contrasts.

Table 1
Mean or median time (s) spent in varied behavioral categories for pairs of rats in a 10-min social interaction trial

Time spent in varied behavioral categories (s)	Vehicle	DZ 3 mg/kg	AEO 10.5 mg/kg	AEO 21 mg/kg	AEO 42 mg/kg
HU test condition					
Sniffing and mutual grooming	41.0±20.4	63.7±30.5	68.2±34.9	119.3±47.6*	102.7±30.2
Adjacent lying	27.7±15.3	77.6±22.5*	34.0±19.7	65.5±25.8	45.8±24.7
Climbing over and crawling under	15.3±4.3	36.3±16.4	25.1±11.3	28.4±7.5	35.3±18.2
Approximation and follow	36.4±19.3	52.7±27.9	47.3±22.9	56.6±28.5	47.9±20.5
Aggressive-type behaviors	25.5±12.1	17.2±11.9	15.4±9.7	5.6±1.8	4.2±2.0*
HF test condition					
Sniffing and mutual grooming	54.3±29.8	60.4±25.4	92.9±36.5	131.4±32.5**	94.3±34.3
Adjacent lying	40.4±27.1	62.7±27.3	34.3±13.5	73.9±25.8*	52.3±22.5
Climbing over and crawling under	13.1±2.8	21.4±7.2	15.0±6.1	15.2±7.3	20.3±9.4
Approximation and follow	43.1±20.7	42.5±18.4	56.5±26.7	47.6±29.4	62.8±30.6
Aggressive-type behaviors	28.6±10.7	16.5±6.2	18.4±7.3	8.1±4.2*	7.9±3.8*

Diazepam (DZ, 3 mg/kg) and angelica essential oil (AEO, 10.5–42 mg/kg) administered PO 40 min before testing. $n=6$ pairs per group. Test conditions were high light, unfamiliar (HU) and high light, familiar (HF). *$P<0.05$, **$P<0.01$, significantly different from vehicle. Bonferroni planned contrasts or Mann–Whitney U-test.

same box for 10 min per day to familiarize them with the apparatus. On the fourth day, the same pairs of rats were once again placed in the test arena for 10 min and the same test procedure was carried out. Pairs of rats were allocated randomly to the following test groups: vehicle control, diazepam (3.0 mg/kg), AEO (10.5, 21 and 42 mg/kg).

2.3.2. Hole-board test

The hole-board apparatus was an open-field arena with four equally spaced holes of 3.5 cm in diameter in the floor, similar to the box used in social interaction test. Rats were placed singly in the centre of the hole-board, and during a 5-min trial the following measures were recorded: the number of head-dips, the time spent head-dipping, the number of rearings, the time spent rearing, the latency to the first head-dipping and the total locomotor activity (numbers of squares crossed). A head dip was scored if both eyes disappeared into the hole (Moreira et al., 2000). Rats were randomly allocated to the following groups: vehicle control, diazepam (0.35, 0.7 and 1.4 mg/kg), AEO (10.5, 21 and 42 mg/kg).

In the above two tests, the behaviors of the animals were recorded with a video camera for behavioral assessment and the rats were observed on a monitor in an adjacent room by an observer who was blind to the drug treatment (File, 1980). The arenas were wiped with a damp cloth after each trial and any faeces removed.

2.4. Statistical analyses

All results were analyzed using one-way analysis of variance (ANOVA) followed by planned contrasts with Bonferroni correction, except in the case where data were not normally distributed and then Mann–Whitney U-tests were employed. P values lower than 0.05 were considered statistically significant.

3. Results

3.1. Social interaction test

In the high light, unfamiliar test condition there was a significant drug-induced increase in social interaction [$F(4, 29)=4.07$, $P<0.05$]. Further analyses confirmed that both 21 and 42 mg/kg doses of AEO significantly increased social interaction time compared with the control group and diazepam (3.0 mg/kg) also markedly enhanced active social interaction ($P<0.01$ or $P<0.05$) (see Fig. 1). In the high light, familiar test condition there was again a significant drug-induced increase in social interaction [$F(4, 29)=3.05$, $P<0.05$], due to the dose of 21 mg/kg of AEO, although diazepam (3.0 mg/kg) had no effect on the total time spent in social interaction (see Fig. 1). The data of varied behavioral categories are shown in Table 1. The results displayed that the increase of social interaction time was due to the enhancing duration of "sniffing and mutual grooming" and/or "adjacent lying" for rats ($P<0.01$ or $P<0.05$).

On the other hand, Mann–Whitney U-tests revealed a significant decrease in the duration of aggressive behaviors

Table 2
Mean (±S.E.M.) locomotor activity score (in squares crossed) for pairs of rats given a 10-min social interaction trial

Drug	Dose (mg/kg)	HU	HF
Vehicle	–	67.4±15.0	51.0±14.3
DZ	3.0	58.2±13.9	50.3±17.2
AEO	10.5	84.3±8.5	80.4±7.8
	21	154.8±15.5***	170.8±9.8***
	42	133.4±13.6**	98.8±19.4*

Diazepam (DZ, 3 mg/kg) and angelica essential oil (AEO, 10.5–42 mg/kg) administered PO 40 min before testing. $n=6$ pairs per group. Test conditions were high light, unfamiliar (HU) and high light, familiar (HF). *$P<0.05$, **$P<0.01$, ***$P<0.001$, significantly different from vehicle. Bonferroni planned contrasts.

Table 3
Measures recorded from rats given a 5-min hole-board test, 40 min after vehicle or drug administration PO

Drug	Dose (mg/kg)	Head-dip latency (s)	Head-dip counts	Head-dip duration	Locomotion (squares crossed)	Rearing counts	Rearing duration
Vehicle	–	131.9±34.2	3.1±0.8	5.2±1.5	28.8±4.2	12.4±1.5	16.3±2.7
DZ	0.35	36.6±7.6**	6.1±0.8*	15.4±3.4*	43.4±4.9	19.0±2.5	24.6±3.7
	0.7	41.7±4.8**	4.2±1.1	10.3±1.4	31.8±5.4	19.2±2.7	19.0±2.6
	1.4	45.3±8.6**	5.1±1.3	11.5±3.0	27.6±6.8	15.3±3.1	22.3±3.0
AEO	10.5	27.2±7.1**	7.7±1.7*	17.6±4.8*	36.0±5.4	13.8±1.6	19.8±3.4
	21	53.8±18.0*	5.1±0.7	11.4±2.3	40.2±8.0	17.2±2.7	21.2±4.0
	42	45.3±11.6**	5.9±1.0	13.4±3.2	36.9±7.0	18.2±3.2	22.2±3.6

Values are median±range, $n=9$ per group. *$P<0.05$, **$P<0.01$, significantly different from vehicle condition. Mann–Whitney U-test.

with AEO at 21 and 42 mg/kg ($P<0.05$ in both HU and HF test conditions). However, diazepam had no effect on aggressive behaviors (see Table 1).

Contrasts with control groups revealed that in the two test conditions, AEO at doses of 21 and 42 mg/kg significantly increased the number of squares crossed in the test chamber, whereas diazepam (3.0 mg/kg) had no effect on locomotor activity (see Table 2).

3.2. Hole-board test

Hole-board measures are summarized in Table 3. ANOVA demonstrated significant treatment effects on head-dip counts [F (6, 62)=2.38, $P<0.05$], head-dip duration [F (6, 62)=2.53, $P<0.05$] and head-dip latency [F (6, 62)=5.40, $P<0.01$]. Further analyses showed that AEO (10.5 mg/kg) and diazepam (0.35 mg/kg) significantly decreased head-dip counts (both $P<0.05$) and head-dip duration (both $P<0.05$). All the test groups significantly shortened head-dip latency compared with the control group ($P<0.01$ or $P<0.05$).

4. Discussion

In accordance with our previous studies, AEO caused an apparent anxiolytic effects in the social interaction and hole-board tests, although different tests were associated with different outcomes.

The social interaction test of anxiety was developed to provide an ethologically based test that was sensitive to both anxiolytic and anxiogenic effects. Generally speaking, an increase in social interaction, without a concomitant increase in locomotor activity, is indicative of an anxiolytic effect, whereas a specific decrease in social interaction indicates an anxiogenic effect. This test provided a new approach to the neurobiological mechanisms underlying anxiety disorders. Ge et al. (1997) has found that the aversive test condition of the social interaction test (HU) increases 5-HT and DA turnover throughout the rat brain. In brief, the social interaction test is an extremely useful animal model for evaluating anxiolytic compounds, which are prescribed for treating social phobia, social failure/impairments and emotional immaturity (Nakamura and Kurasawa, 2001).

In the social interaction test, AEO decreased aggressive behaviors at the doses of 21 and 42 mg/kg, while the doses of 21 and 42 mg/kg significantly increased social interaction time of the high light, unfamiliar test condition and 21 mg/kg could also prolong social interaction time of the high light, familiar test condition. The interesting thing is that under a high light, familiar condition, 21 mg/kg of AEO was effective, but not 10.5 and 42 mg/kg. Obviously, AEO exhibits an inverted U-shaped dose–response curve here, which is consistent with its axiolytic effect in three assays predictive of anxiolytic activity in male mice: elevated plus-maze, light/dark and stress-induced hyperthermia tests (Chen et al., 2004). In addition, the 21 and 42 mg/kg doses of AEO that increased social interaction also increased the number of squares crossed in the social interaction test arena. It could be argued that the increased social interaction in rats received AEO treatment is merely an artifact of the hyperactivity induced by the drug. However, the data of varied behaviors of rats in the present study indicated that the increased social interaction elicited by 21 and 42 mg/kg AEO was originated from the increase in sniffing, grooming and adjacent lying rather than moving behaviors. Thus, the increased social interaction occurred during relatively "static" behaviors such as sniffing and adjacent lying, but not when the rats were involved in locomotion such as climbing over, crawling under, following or approximation. Additionally, since locomotor activity of the rats tested in the hole-board arena did not change, we presumed that the hyperactivity in social interaction test might due to the interference between a pair of rats.

The hole-board test has been widely used to assess emotionality, anxiety and/or responses to stress in animals (Rodriguez Echandia et al., 1987). Several behaviors can be readily observed and quantified in the test, which makes a comprehensive description of the animals' behavior possible. It has been established that head-dipping behavior in mice and rats reflects exploration distinct from general locomotor activity (File, 2001). Based on previous reports, Takeda et al. (1998) indicated that head-dipping behavior was sensitive to changes in the

emotional state of the animal, and suggested that the expression of an anxiolytic state in animals might be reflected by an increase in head-dipping behavior. Our results are consistent with previous reports of an increase in the frequency and duration of exploratory head-dips in rat which received oral administration of non-sedative dose (0.35 mg/kg in this study) of diazepam on a hole-board. This model, as noted above, also yielded a consistent anxiolytic action of AEO, although it was at the lowest dose (10.5 mg/kg). It seems that future behavioral studies of AEO in rats could usefully explore even lower doses of the drug.

It is obvious that there are some differences between the outcomes of the two tests, such as the locomotor activity of the tested animals and the anxiolytic doses of AEO and diazepam. Although we do not know the reasons for the differences, the two tests are quite different, and File and Pope (1974) found that drug effects that are present when one rat is tested alone in the hole-board may not be present when two rats are tested together. This may be because that the behavior of one rat influences that of the other. Both File (1992) and Belzung and Le Pape (1994) have reported that different measures of anxiety (e.g. as recorded in the plus-maze, social interaction, Vogel conflict, light/dark exploration, hole-board, free-exploration, and neophobia tests) correlated very poorly with one another. Indeed, they can actually yield separate anxiety factors (e.g. plus-maze anxiety, social interaction anxiety, Vogel anxiety; File, 1992), thereby conforming the growing view that the inherent inconsistency exists in the tapping into different facets of anxiety.

In summary, this research expanded previous findings with AEO to show consistent anxiolytic effects in both the social interaction and hole-board tests which would further substantiate prediction of clinical efficacy of the agent. For example, since there is a significant decrease in the duration of aggressive behaviors of rats with AEO in social interaction test, the volatile oil might be a novel therapeutic approach to reduce or inhibit heightened aggressiveness and possibly to treat aggressive behavior associated with psychiatric disorders.

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