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The effects of angelica essential oil in three murine tests of anxiety

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Abstract

The effects of angelica essential oil in three assays predictive of anxiolytic activity in male mice were studied, with diazepam as a positive anxiolytic control. In the elevated plus-maze test, compared to the positive control diazepam, angelica essential oil (30.0 mg/kg, PO) had a modest anxiolytic-like effect (increased the percentage of open-arm time and reduced the percent protected head dips). In the light/dark test, angelica essential oil (30.0 mg/kg) prolonged the time spent in the light area without altering the locomotor activity of the animals. In the stress-induced hyperthermia test, 60 and 70 min after drug administration, rectal temperature was measured twice, angelica essential oil at the dose of 30.0 mg/kg inhibited stress-induced hyperthermia. Thus, these findings indicate that angelica essential oil, as does diazepam, exhibits an anxiolytic-like effect. Further studies will be required to assess the generality of the present findings to other species and behavioural paradigms.

Keywords: Angelica essential oil; Anxiolytic; Elevated plus-maze; Light/dark test; Stress-induced hyperthermia; Mouse

1. Introduction

Chinese Angelica, "Dong Quai" is the most important female tonic remedy in Chinese medicine. Dong Quai is the Chinese name of the root of the plant *Angelica sinensis* belonging to the family Umbelliferaceae. It is related to the European and Japanese Angelica, but its medicinal actions are more potent. The extract of Chinese Angelica root is believed to improve gynecological diseases, such as menoxenia and anemia, in the clinic via its hemogenic analgesic and sedative activities. These effects are thought to be due to components of the volatile oil, particularly ligustilide. Ligustilide, butylene phthalide and butyl phthalide are the major components of the essential oil (Wang et al., 1998). It has been reported that ligustilide inhibited contractions of isolated uteri from various animal models (Mei et al., 1991; Pi, 1955). Furthermore, intraperitoneal administration of ligustilide (0.14 ml/kg body weight) to guinea pigs inhibited asthmatic reactions induced by acetylcholine and histamine. Ligustilide (32.5–130.0 μl/ml) also inhibited smooth muscle contractions induced by barium sulfate, acetylcholine and histamine in isolated guinea pig trachea (Tao et al., 1984). Moreover, Matsumoto et al. reported that ligustilide had a beneficial effect on psychological stress-induced pathophysiological changes in the central nervous system function. For example, it could reverse the decrease in the duration of pentobarbital sleep in mice caused by social isolation stress and activation of central nonadrenergic systems (Matsumoto et al., 1998). Yang et al. demonstrated that ligustilide exhibited a sedative activity stronger than succinic acid (Yang et al., 1986).

Different drugs, known for their sedative properties at high doses, such as minor tranquillizers, also exhibit an anticonflict effect. In this study, a possible anxiolytic property of angelica essential oil was investigated by using three murine tests of anxiety: elevated plus-maze test, light/dark test and stress-induced hyperthermia test. The elevated plus-maze is one of the most extensively used models for the investigation of drug effects on anxiety-related behaviour in laboratory rodents. It is based on the aversion of

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rodents for open spaces, and anxiolytics have been found to increase the proportion of time spent on the open arms (Rodgers and Johnson, 1995). The light/dark test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors, that is, novel environment and light (Crawley and Goodwin, 1980). Anxiolytics have been found to increase locomotion and time spent in the light zone, whereas anxiogenics decrease them (Imaizumi et al., 1994). Stress-induced hyperthermia in singly housed male mice appears to be a robust, reproducible and easy paradigm to study putative anxiolytic effects of drugs (Van der Heyden et al., 1997). In this paradigm, singly housed mice are subject to two sequential rectal temperature measurements with a 10-min interval. The first measurement is the basal temperature ($T1$). Because of the stress induced by handling and rectal probe insertion, the value at the second temperature recording ($T2$) exceeds the value of the initial measure. The difference (ΔT) is defined as reflecting stress-induced hyperthermia (Olivier et al., 1998). Benzodiazepine receptor agonists, alcohol and 5-HT_{1A} receptor agonists have been reported to decrease stress-induced hyperthermia, suggestive of anxiolytic-like efficacy (Olivier et al., 2003). Because the three tests have been validated pharmacologically, behaviourally and physiologically, as models of experimental anxiety, we use them to validate the putative anxiolytic effect of angelica essential oil, and meanwhile, diazepam treatment was included as a positive control.

2. Methods

2.1. Animal

Male Swiss mice (Experimental Animal Center of Shenyang Pharmaceutical University) weighing 20–22 g were used. For elevated plus-maze and light/dark tests, mice were maintained under a 12-h reversed light cycle (lights off 07:00), and five animals were housed in a cage (25×14×14 cm). For stress-induced hyperthermia test, mice were housed in groups of 10 animals per cage (50×25×20 cm) under nonreversed 12L/12D cycle conditions (lights on 07:00), and the animals were individually housed in smaller cages (25×14×14 cm) 24 h before testing. All the animals were housed at constant room temperature (21±2 °C) and relative humidity (60±10%) with free access to food and water except during the test period. All subjects were experimentally naïve. Experiments were carried out at least 1 week after the arrival of the animals.

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 (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 a 3% aqueous Tween 80 solution. All drugs were prepared immediately before use and were given orally in a volume of 10 ml/kg body weight. Control mice received 3% aqueous Tween 80 solution only. The effects of the drugs were estimated 40 (for elevated plus-maze and light/dark tests) or 60/70 (for stress-induced hyperthermia test) minutes after administration. Drug dose, pretreatment time and selection of 3% Tween 80 solution as vehicle were based on findings in preliminary experiments or taken from the literature.

2.3. Procedures

2.3.1. Elevated plus-maze test

The test procedure and scoring methodology for elevated plus-maze test have been described in detail elsewhere (e.g., Rodgers and Johnson, 1995). In brief, the apparatus was composed of two open (30×5×0.25 cm) and two enclosed (30×5×15 cm) arms that radiated from a central platform (5×5 cm) to form a plus sign. The plus-maze was elevated to a height of 45 cm above floor level by a single central support. The maze floor was constructed of black Plexiglas; the side and end walls of the enclosed arms were made of clear Plexiglas. A slight raised edge on the open arms (0.25 cm) provided additional grip for the animals, whereas open-arm activity was further encouraged by testing under dim red light (4×25 W).

The experiment was conducted during the dark phase of the light cycle (10:00–14:00 h). To facilitate adaptation to new surroundings, mice were transported to the laboratory at least 1 h prior to testing. The trial was started by placing an animal on the central platform of the maze facing an open arm. A standard 5-min test duration was used, and, between subjects, the maze was thoroughly cleaned with damp and dry towels. Test sessions were recorded by an overhead video camera linked to a monitor and video recorder in an adjacent laboratory.

Behaviours, scored off videotape by a trained observer, comprised both conventional and ethological parameters (Rodgers and Johnson, 1995), i.e., indices of anxiety (percentage open-arm entries, percentage open-arm time and percentage protected forms of head dip, percentage stretched attend posture and frequency of end exploring), risk assessment (total stretched attend postures), locomotion (closed and total arm entries), vertical activity (rearing frequency) and exploration (total head dips). Head dips and stretched attend postures were differentiated as “protected”

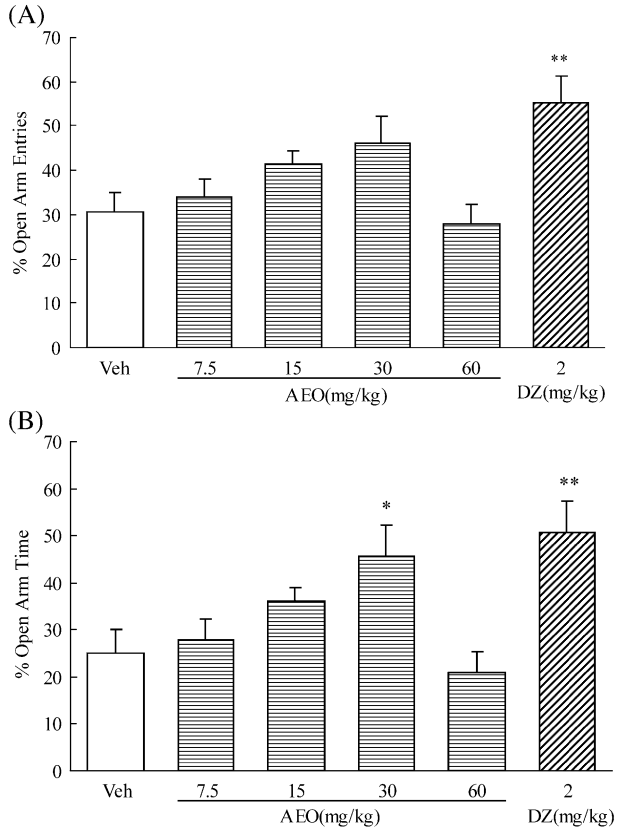

Fig. 1. Effects of angelica essential oil (AEO) and diazepam (DZ) in the elevated plus-maze in male mice (n=9–10). Results are expressed as means±S.E.M. The following parameters are shown: percent open-arm entries [percentage of entries into open arms with respect to total entries into the arms (A)]; percent open-arm time [percentage of time spent in open arms with respect to total time spent in the arms (B)]. Mice were given a 5-min test 40 min after PO with DZ (2 mg/kg) or AEO (7.5, 15, 30 and 60 mg/kg). Significant differences vs. the vehicle-treated control group were calculated with two-tailed Dunnett's t-tests (*P<0.05, **P<0.01) following significant one-way ANOVA. See Table 1 for complementary data.

(i.e., occurring on or from the relative security of the central platform/closed arms) and "unprotected" (i.e., occurring on or from the open arms).

2.3.2. Light/dark test

The dimensions of the compartment for light/dark test are two-fifths for the dark compartment and three-fifths for the light compartment with a size of 45×27×27 cm, as described by Costall et al. (1989). The light chamber was painted white and was illuminated brightly with a 60-W light source (400 Lux), and the dark chamber was painted black, screened from ambient light and illuminated by red light (0 Lux). The red and white lights were located 17 cm above the box. The box was open-topped, and the base was lined into 9-cm squares. The compartments were connected by an opening 7.5×7.5 cm, located at floor level in the center of the partition. In the experiment, mice were taken from a dark holding room in a dark container to the dark testing room. After a 1-h period of adaptation to the new environment, each mouse was tested by placing it in the center of the light area, facing away from the dark one, and was allowed to explore the novel environment for 5 min. The number of transfers from one compartment to the other, the time spent in the illuminated side and the number of line crossings in the light and the dark sections were measured. A mouse was considered to have entered the new area when all four legs crossed the threshold into the compartment. The box was cleaned between trials.

The video tapes from the behavioural studies were evaluated by a "blind" reviewer, who was given a standard evaluation form.

2.3.3. Stress-induced hyperthermia test

The test procedure for stress-induced hyperthermia in singly housed mice was adapted from Spooren et al. (2002). Rectal temperature was measured by inserting a thermometer probe (model Jm 624u, Liwen electronic) for a length of 2 cm into the rectum of the mouse. Digital recording of the temperature was determined with an accuracy of 0.1 °C. The probe, dipped into liquid paraffin before insertion, was held in the rectum until a stable rectal temperature had been obtained for 20 s. For each individual mouse, a basal "nonstressed" temperature 60 min after drug treatment (T1),

Table 1
Effects of angelica essential oil (AEO) and diazepam (DZ) on plus-maze behaviour in male mice

Behaviour	VEH	DZ 2.0 (mg/kg)	AEO (mg/kg)				F(5,57)
			7.5	15.0	30.0	60.0	
Total arm entries	16.8±1.8	26.6±2.4**	15.4±1.2	16.3±1.7	17.6±2.0	18.2±1.9	5.03, P<0.01
Open-arm entries	5.1±0.8	15.5±2.7**	5.4±0.8	6.8±0.8	8.4±2.0	5.4±1.1	6.77, P<0.01
Closed-arm entries	11.7±1.5	11.1±1.3	10.0±0.7	9.6±1.1	9.1±1.0	12.8±1.1	1.45, NS
Total rears	15.3±1.4	16.4±3.3	13.9±1.8	12.1±1.6	13.1±1.9	14.7±1.9	0.53, NS
Total head dips	22.2±3.6	31.0±4.9	18.0±3.7	23.4±3.3	26.4±6.1	14.3±1.7	2.19, NS
%p Dips	60.7±6.8	24.4±4.8**	46.6±7.0	38.8±6.5	30.6±7.4**	68.3±6.0	7.15, P<0.01
Total SAP	4.7±1.1	2.8±1.4	3.6±0.5	3.6±0.7	3.0±0.8	2.8±0.7	0.62, NS
%p SAP	59.9±12.4	15.0±10.0*	73.8±7.8	41.1±15.1	41.1±12.5	41.1±11.7	3.03, P<0.05
End exploring	1.6±0.6	8.0±2.5**	1.7±0.6	2.2±0.6	3.6±1.5	1.1±0.6	4.05, P<0.01

SAP, stretched attend posture; %p, percent protected. Values represent means±S.E.M. from 9 to 10 mice. Dunnett's t-tests after one-way ANOVA. See Fig. 1 for complementary data.

* Significance of difference: P<0.05 compared with vehicle condition.

** Significance of difference: P<0.01 compared with vehicle condition.

a "stressed" temperature 70 min after drug treatment ($T2$) and the difference ($\Delta T = T2 - T1$) were determined. All experiments were performed between 8 and 12 a.m.

2.4. Statistical analyses

All the data given represent mean±S.E.M. values. Data were analyzed by means of analysis of variance (ANOVA). Whenever ANOVA was significant, further multiple comparisons were made using the Dunnett's t-tests. All analyses were performed using the software SPSS V11.5 for windows. The level of statistical significance adopted was $P<0.05$.

3. Results

3.1. Elevated plus-maze test

Data are summarized in Fig. 1 and Table 1. ANOVA (df =5, 57) indicated significant treatment effects on total arm entries (F=5.03, $P<0.01$), open-arm entries (F=6.77, $P<0.01$), percent open-arm entries (F=4.84, $P<0.01$), percent open-arm time (F=5.31, $P<0.01$), percent protected head dips (F=7.15, $P<0.01$), percent protected stretched attend postures (F=3.03, $P<0.05$) and end exploring (F=4.05, $P<0.01$). Further analyses confirmed that diazepam (2 mg/kg) significantly increased total ($P<0.01$) and open ($P<0.01$)-arm entries without altering closed-arm entries. Percent open-arm entries ($P<0.01$), percent time spent on the open arms ($P<0.01$), were also significantly increased. On the ethological measures, diazepam treatment exhibited a reduction in percent protected head dips ($P<0.01$), percent protected stretched attend postures ($P<0.05$) and an increase in end exploring ($P<0.01$). As for angelica essential oil, post hoc analysis revealed that 30 mg/kg dosage, but not any other dose, significantly increased the time spent on the open arm ($P<0.05$) and reduced percent protected head dips ($P<0.01$).

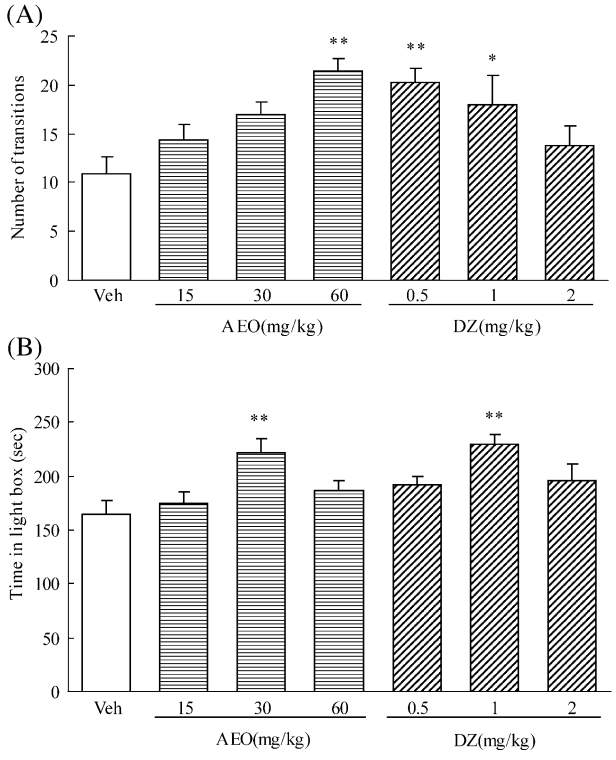

Fig. 2. Effects of angelica essential oil (AEO) and diazepam (DZ) in the light/dark test in male mice (n=10). (A) The number of transitions; (B) time spent in the light box. Results are expressed as means±S.E.M. Mice were given a 5-min test 40 min after PO with DZ (0.5, 1, 2 mg/kg) or AEO (15, 30 and 60 mg/kg). Significant differences vs. the vehicle-treated control group were calculated with two-tailed Dunnett's t-tests (*$P<0.05$, **$P<0.01$) following significant one-way ANOVA. See Table 2 for complementary data.

Table 2
Effects of angelica essential oil (AEO) and diazepam (DZ) on locomotion in mice in the light/dark test

Treatment	Dose (mg/kg)	Number of line crossings in light area	Number of line crossings in dark area
Vehicle	–	36.4±5.3	31.0±5.7
AEO	15	50.6±4.8	43.3±3.7
	30	46.6±4.8	33.5±5.2
	60	66.1±11.3*	45.6±4.0
DZ	0.5	64.3±6.9*	47.2±3.4
	1.0	65.9±10.4*	40.7±7.8
	2.0	47.2±5.4	38.0±2.9

Values represent means±S.E.M. from 10 mice. Significance of difference: **$P<0.01$ compared with vehicle condition; Dunnett's t-tests after one-way ANOVA. See Fig. 2 for complementary data.
* $P<0.05$.

3.2. Light/dark test

Analysis of the results are represented in Fig. 2 and Table 2. ANOVA (df =6, 69) indicated significant treatment effects on the time in light box (F=4.23, $P<0.01$), number of transitions (F=4.04, $P<0.01$) and the number of line crossings in the light area (F=2.46, $P<0.05$). Further analyses confirmed that angelica essential oil (30 mg/kg) and diazepam (1 mg/kg) significantly increased the time in light box (all $P<0.01$). When compared to vehicle condition, angelica essential oil (60 mg/kg) and DZ (0.5 and 1.0 mg/kg) significantly increased the transitions ($P<0.01$ or $P<0.05$). Diazepam, at doses of 0.5 and 1.0 mg/kg, but not at 2 mg/kg, and angelica essential oil, at the dose of 60 mg/kg, but not following 15 or 30 mg/kg, also significantly increased the number of line crossings in the light area during the 5-min test (all $P<0.05$).

3.3. Stress-induced hyperthermia test

The effects of drugs on basal body temperature and stress-induced hyperthermia are shown in Fig. 3. ANOVA (df=4, 49) indicated significant treatment effects on the stress-induced hyperthermia ΔT (F=5.09, $P<0.01$). Post

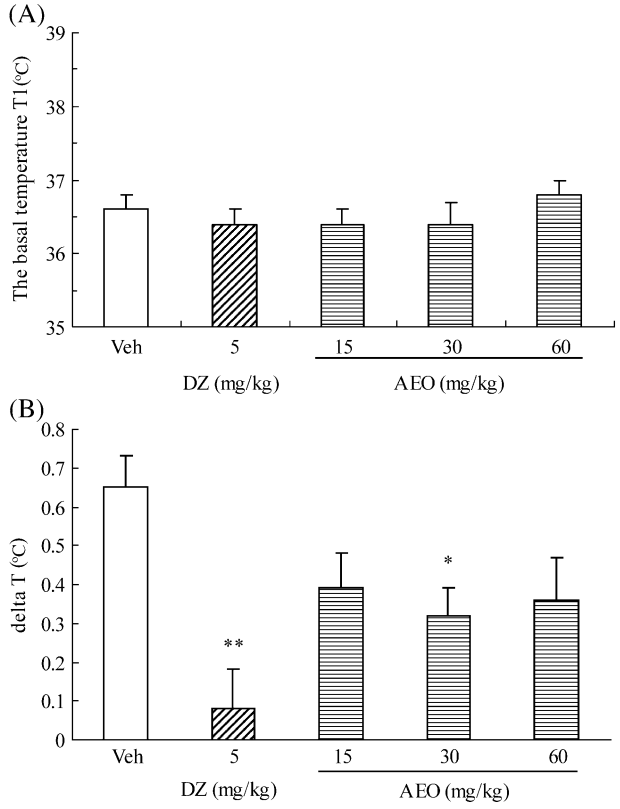

Fig. 3. Effects of diazepam (DZ, 5.0 mg/kg) and angelica essential oil (AEO, 15, 30 and 60 mg/kg) in the stress-induced hyperthermia paradigm on the basal temperature measurement T1 (A) and the stress-induced hyperthermia ΔT (B) in singly housed male mice (n=10 per dose). Significant differences vs. the vehicle-treated control group were calculated with two-tailed Dunnett's t-tests (*P<0.05, **P<0.01) following significant one-way ANOVA.

hoc comparisons versus the vehicle-treated control group indicated that stress-induced hyperthermia was significantly attenuated following 5.0 mg/kg diazepam and 30 mg/kg angelica essential oil [P<0.01 and P<0.05, respectively, as shown in (B)]. Neither of the two compounds affected basal core body temperature $T1$ at any of the doses tested [as shown in (A)].

4. Discussion

We here demonstrated that the 30 mg/kg oral dose of angelica essential oil produced a significant anxiolytic-like effect in three anxiety models (elevated plus-maze, light/dark test and stress-induced hyperthermia paradigm) in mice.

Conventional anxiety indices in the elevated plus-maze test comprise percentage open-arm entries and percentage time spent in these areas in the maze, with anxiolytics generally increasing and anxiogenics decreasing these measures. More recently, it has been argued that the incorporation of a range of ethological parameters may enhance the utility of this paradigm (Rodgers and Johnson, 1995). In agreement with previous findings (Kuribara et al., 1998; Lepicard et al., 2000), diazepam at 2 mg/kg produced a robust anxiolytic-like action under present test conditions, with significant increases in percent open-arm entries, percent open-arm time and end exploring paralleled by a reduction in percent protected stretched attend postures and percent protected head dips. The increased total entry score in animals treated with diazepam is due to an increase in open-arm activity only. Diazepam did not have an effect on closed-arm entries. Although locomotor activity is often assessed by total arm entries, they arguably do not reflect exclusively a locomotor influence. Factor analytic studies have revealed that while closed-arm entries load only on an "activity" factor, total arm entries load on both "anxiety" and "activity" (Rodgers and Dalvi, 1997). The results of this study indicate that 30 mg/kg angelica essential oil produces an anxiolytic-like effect on both traditional and ethological indices, although somewhat weaker than those caused by 2 mg/kg diazepam, i.e., angelica essential oil did not alter the percentage of open-arm entries. As in previous studies with both anxiolytic and anxiogenic manipulations (Rodgers et al., 1992; Cole and Rodgers, 1993, 1994), measures of risk assessment appeared to be more sensitive to drug action than the traditional indices. Thus, reductions in the percentage of protected stretched attend postures and in the percentage of protected head dips are of great value for assessing the anxiolytic effects of diazepam and angelica essential oil.

Good agreement has been observed between relative potency of drugs clinically used in the treatment of anxiety in humans and their ability to facilitate exploratory activity in the light/dark paradigm in mice (Crawley, 1981). Transitions have been reported to be an index of activity exploration because of habituation over time, and the time spent in each compartment to be a reflection of aversion (Belzung et al., 1987). In this study, analysis of locomotor activity demonstrated that 0.5 and 1.0 mg/kg diazepam-treated and 60 mg/kg angelica essential oil-treated animals were more active in the light box. On the other hand, diazepam at 0.5 and 1.0 mg/kg and angelica essential oil at 60 mg/kg also increased the number of transitions, while only 30 mg/kg angelica essential oil and 1.0 mg/kg diazepam prolonged the time spent in the light box. Young and Johnson (1991) concluded that simply the measurement of the time spent in the light area, but not the number of transfers, was the most consistent and useful parameter for assessing anxiolytic-like action. Furthermore, Lepicard et al. (2000) reported that the time spent in the light was a stronger indication in the study of anxiety, whereas the number transfers reflected both anxiety and exploration. These observations seem to be in good agreement with our results.

Psychological stress, such as noise, heat, handling, novel environment and forced exercise, produces an acute increase in body temperature in various species, including the mouse, rat, rabbit and human (Borsini et al., 1989; Lecci et al.,

1990; Marazziti and Di Muro, 1992; Zethof et al., 1995). In many anxiety disorders, it occurs as an integral part of the pathology and is often considered a representative symptom of the disease, e.g., in generalized anxiety as classified in DSM-IV (Olivier et al., 2003). The stress-induced hyperthermia in singly housed male mice is a reliable experimental paradigm in anxiety and psychological stress research (Olivier et al., 1998). Furthermore, because the parameter measured for anxiety in the stress-induced hyperthermia procedure is not dependent on locomotor activity, like in almost all other anxiety tests, and in addition, it is a physiological measure that potentially may reflect other (autonomic) aspects involved in anxiety and fear processes, the stress-induced hyperthermia procedure is an extremely valuable addition to tests in the anxiety field (Olivier et al., 2003). In this study, 30 mg/kg angelica essential oil, similar to 5 mg/kg diazepam, had an anxiolytic-like effect as reflected by a selective decrease in stress-induced hyperthermia (no intrinsic effect on basal temperature was seen).

The pharmacological mechanism that might account for the anxiolytic effect of angelica essential oil has yet to be determined. It had been reported that ligustilide and butylene phthalide, components of *Angelica sinensis*, attenuated the suppressive effects of the benzodiazepine inverse agonist, FG 7142, on pentobarbital sleep in group-housed mice (Matsumoto et al., 1998). The findings of Matsumoto et al. (1998) suggest that the $GABA_A$ receptor system is at least partly involved in the pharmacological activity of ligustilide and butylene phthalide.

In summary, angelica essential oil, at some of the tested doses, exhibited an anxiolytic-like effect in the elevated plus-maze, the light/dark box and the stress-induced hyperthermia paradigms in male Swiss mice. Such data may thus indicate that angelica essential oil might have some anxiolytic properties.

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