Behavioral Effects of Plant-Derived Essential Oils in the Geller Type Conflict Test in Mice

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ABSTRACT—The present study was conducted to further explore plant-derived essential oils that possess an anticonflict effect using the Geller type conflict test in ICR mice. The benzodiazepine anxiolytic diazepam increased the response (lever pressing) rate during the alarm period (i.e., an anticonflict effect), but the 5-HT_{1A} partial agonist buspirone did not. Oils of juniper, cypress, geranium and jasmine did not produce any effect in this test. Frankincense oil decreased the response rate during the safe period at 1600 mg/kg, but did not exhibit any effect on the response rate during the alarm period. In contrast, lavender oil increased the response rate during the alarm period in a dose-dependent manner in the same manner as diazepam. These results indicate that not only rose oil but also lavender oil possess an anticonflict effect in mice.

Keywords: Geller type conflict test, Anxiety, Plant-derived essential oil

Various plant-derived essential oils (EOs) have traditionally been used in Europe in the treatment of a variety of illnesses. The medicinal use of EOs began in the ancient Egyptian Era and has been practiced ever since. The "aromatherapy" movement (1) has spread worldwide, despite the lack of a scientific basis for the effectiveness of EOs. On the other hand, the long history of EOs in therapy suggests that they may indeed be effective, and for this reason, the author hypothesized that EOs possess pharmacological effects.

I previously examined the effects of four kinds of EOs, including rose, ylang-ylang, camomile and orange oils, using two types of conflict tests, namely the Vogel type and the Geller type, in mice (2). The study revealed that rose oil (ROS) produced significant anticonflict effects in both tests, suggesting that ROS possesses a pharmacological action similar to anxiolytics. One of the remaining questions is whether only ROS possesses such a pharmacological action. Therefore, the present study was conducted to explore whether other EOs possess an anticonflict effect using the Geller type conflict test in mice.

Male ICR mice (Clea Japan, Tokyo) were used for the experiment. The mice were 7-week-old and weighed between 33 – 37 g at the start of the experiment. This study was conducted using the Geller type conflict test, which is a standard method for evaluating anxiolytics (3), in order to test the antianxiety-like effect of certain EOs. First, animals were subjected to food deprivation in order to induce hunger. Then, they were trained under a previously reported (2, 4) MULT FR20/FR20-punishment schedule of food reinforcement, using the apparatus for the Geller type conflict test (GT-8510, GT-8005 and GT-7715; O’hara & Co., Tokyo), which has also been reported previously (2, 4-7). The schedule consisted of four pairs of an alternating safe period of 5 min and an alarm period of 5 min. During the safe period, the mouse’s lever pressing was reinforced by food pellets at FR20 without electric shock. During the alarm period, which was indicated by a warning stimulus (tone signal: 800 Hz, 90 dB), every 20th lever press was punished using an electric shock (50 – 90 V, ca. 0.3 mA, 50 Hz AC, duration = 0.3 s). After establishment of stable baseline responses for the safe and alarm periods, animals showed a high response rate during the safe period and a low response rate during the alarm period. Subsequently, challenge testing sessions were conducted at intervals of 3 – 4 days in which drugs were administered to animals 20 min before the start of the test session. Response rates were recorded separately for the safe and the alarm periods. On non-experimental days, animals were trained without any treatment, and the stability of the behavioral baseline was assessed. All experiments in this study were performed in accordance with the Ethics Committee for Experimental Animals of the National Institute for Environmental Studies.

The tested drugs were the benzodiazepine anxiolytic, diazepam (DZ) (Cercine Inj., Takeda Chem. Ind., Osaka, and the 5-HT_{1A} partial agonist buspirone (BUS) (Research Biochemicals, Natic, MA, USA). DZ was diluted in 10%
propylene glycol (10%PG) solution, and BUS was dissolved in physiological saline (0.9% NaCl solution). The EOs used in this study were frankincense (FRA), juniper (JUN), cypress (CYP), geranium (GER), jasmine (JAS) and lavender (LAV), which were extracted from Boswellia thurifera, Juniperus communis, Cupressus sempervirens, Pelargonium adoratissimum, Jasminum officinale and Lavandula angustifolia, respectively (1). All of the samples were produced by Maggie Tisserand, Ltd. (Brighton, UK) for use in aromatherapy. The oils tested were diluted with olive oil (Wako Pure Chemical Ind., Osaka). All injection volumes were 1 ml/100 g body weight, and the injections were made subcutaneously in the case of DZ and BUS and intraperitoneally in the case of EOs.

The data obtained in the experiments were first analyzed by one way analysis of variance (ANOVA), followed by Dunnett’s test. A 5% level of significance was used.

As previously reported (2, 5, 8), DZ produced a significant effect in the Geller type conflict test in ICR mice (Fig. 1a). The response rate during the safe period significantly increased in response to DZ administration (F(3, 76) = 2.92, P < 0.05). The Dunnett’s test results revealed that DZ at 0.5 mg/kg significantly increased the response rate when compared with that of vehicle (10% PG) administration. The response rate during the alarm period dramatically increased by DZ, demonstrating that DZ produced a significant anticonflict effect in the test (F(3, 76) = 3.69, P < 0.05). Thus, I confirmed that it is possible to use this test in mice to assess the anxiolytic-like effect of chemicals. On the other hand, BUS did not produce an anticonflict effect, as previously reported (8 – 10). In particular, the response rate during the alarm period did not change (F(3, 32) = 0.18, P > 0.05), whereas the response rate during the safe period decreased significantly (F(3, 32) = 3.68, P < 0.05) (Fig. 1b).

FRA produced a significant behavioral effect. FRA at 1600 mg/kg significantly decreased the response rate during the safe period (F(3, 90) = 3.75, P < 0.01) (Fig. 2a). However, it did not produce any effect on the response rate during the alarm period (F(3, 90) = 0.25, P > 0.05) (Fig. 2a).

JUN, CYP and GER did not produce any effect on the response rate during either the safe period (JUN, F(3, 72) = 0.19, P > 0.05; CYP, F(3, 64) = 0.18, P > 0.05; GER, F(3, 56) = 1.07, P > 0.05) or the alarm period (JUN, F(3, 72) = 0.72, P > 0.05; CYP, F(3, 64) = 0.45, P > 0.05; GER, F(3, 56) = 0.05, P > 0.05) (Fig. 2b, 2c, 2d, respectively).

JAS did not produce a significant effect on the response rate during the safe period (F(3, 64) = 0.17, P > 0.05) (Fig. 2c). Although JAS tended to increase the response rate during the alarm period, the difference was not statistically significant (F(3, 64) = 0.53, P > 0.05). In contrast, LAV increased the response rate during the alarm period significantly.

![Fig. 1](image-url)  
Fig. 1. Effects of diazepam (a) and buspirone (b) in the Geller type conflict test in ICR mice. The upper panel of each figure shows the response (lever pressing) rate during the safe (unpunished) period, while the lower panel shows the response rate during the alarm (punished) period. Points indicate mean values, while vertical lines indicate S.E.M. n = 20 (a), n = 9 (b) for each point. Significant differences compared with vehicle-treated control values (*P < 0.05, **P < 0.01; Dunnett’s test (two-tailed).
Dose (mg/kg, i.p.)

Fig. 2. Effects of frankincense oil (a), juniper oil (b), cypress oil (c), geranium oil (d), jasmine oil (e) and lavender oil (f) in the Geller type conflict test in ICR mice. Data are shown as in Fig. 1. n = 16 (a), n = 13 (b), n = 14 (c), n = 15 (d), n = 17 (e), n = 15 (f).

\(F_{(4, 70)} = 2.86, P < 0.05\) in a dose-dependent manner (Fig. 2f); that is, LAV exhibited a significant anticonflict effect in this test. LAV also produced a significant effect on the response rate during the safe period at 1600 mg/kg \(F_{(4, 70)} = 4.3, P < 0.01\).

The present study revealed that of the six EOs tested, JUN, CYP, GER and JAS did not produce an anticonflict effect in the Geller type conflict test. Although FRA decreased the response rate during the safe period, it did not increase the response rate during the alarm period at a dose as high as 1600 mg/kg. Thus I concluded that FRA does not produce an anticonflict effect in this test, either.

The most important finding in the present study is that LAV increased the response rate during the alarm period, showing that LAV has an anticonflict effect like the benzodiazepine anxiolytic DZ. This result provides further evidence in support of the hypothesis that EOs possess pharmacological actions. A previous study (2) demonstrated that ROS has anticonflict effects in both the Vogel type and Geller type conflict test. Thus, more than one of the EOs has been shown to possess anticonflict effects, suggesting that other EOs with an anticonflict effect may be found among unexamined EOs. Based upon results of the present and previous studies, certain EOs likely cause an antianxiety effect via their pharmacological action.

The present study confirmed that BUS, a 5-HT\textsubscript{1A} partial agonist, does not produce an anticonflict effect in the Geller type conflict test, suggesting that the 5-HT\textsubscript{1A} receptor does not play an important role in a mechanism that underlies the anticonflict effect of LAV. In contrast, the GABA\textsubscript{A} receptor, which includes a benzodiazepine binding site, is likely involved in an action mechanism of LAV given that the benzodiazepine agonist DZ produces an anticonflict effect similar to LAV, as confirmed in the present study. In addition, a non-benzodiazepine mechanism is also probable as a mechanism for the anticonflict effect of LAV. For example, another EO ROS also produces a significant anticonflict effect; however, the effect is not antagonized by the benzodiazepine antagonist flumazenil (2), showing that ROS produces an anticonflict effect via a non-benzodiazepine mechanism. It is notable that LAV contains a number of chemicals (11). Therefore, more
than one of the constituent elements of LAV likely produce an anticonflict effect via plural mechanisms. Thus, pharmacologically active constituent elements of LAV should be examined in future research to clarify mechanisms that underlie the anticonflict effect of LAV.

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