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Anticonflict Effects of Plant-Derived Essential Oils

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UMEZU, T. *Anticonflict effects of plant-derived essential oils.* PHARMACOL BIOCHEM BEHAV **64**(1) 35–40, 1999.—The present study examined the pharmacological actions of four different plant-derived essential oils (rose, ylang-ylang, camomile, orange) in two types of conflict tests using ICR mice. In the Vogel conflict test, in which any drinking behavior of the mice was punished by an electric shock, the benzodiazepine agonist, diazepam (DZ), increased the number of electric shocks the mice received. This number increased after administration of rose oil. In contrast, ylang-ylang, camomile, and orange oil did not produce such an effect in this test. In the Geller conflict test where lever-pressing of mice was reinforced by food pellets and then punished by electric shock, response (lever-pressing) rate during the alarm period was increased as well by the positive control drug, DZ. Similarly, the response rate during the alarm period increased after administration of rose oil. Here as well, ylang-ylang, camomile, and orange oils did not produce an anticonflict effect. In the Vogel conflict test, the anticonflict effect of DZ was reversed by the benzodiazepine antagonist, flumazenil (Ro15-1788) (FL). However, the effect of rose oil in this test was not antagonized by FL. The present study showed that rose oil possesses anticonflict effects, and that the effects are not mediated by the benzodiazepine binding site of the $GABA_A$ receptor complex. Such pharmacological actions may at least partially account for human behavioral effects attributed to essential oils. © 1999 Elsevier Science Inc.

Vogel conflict test Geller conflict test Anticonflict effect Diazepam Rose oil Ylang-ylang oil Camomile oil Orange oil Mice

VARIOUS plant-derived essential oils (EOs) have traditionally been used in Europe in the treatment of a variety of different illnesses. The medicinal use of EOs began in the ancient Egyptian Era, and has been practiced ever since. The "aromatherapy" movement (18) has spread world-wide, 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. EOs are believed to be effective in treating mental disorders (18); for example, it is believed that EOs, such as rose, ylang-ylang, camomile, jasmine, and lavender are effective in relieving anxiety.

Conflict paradigms, where animals must choose whether to accept reinforcers such as food and water when they are accompanied by punishment such as electric shock, have facilitated preclinical evaluation of putative antianxiety drugs given that these methods exhibit good predictive validity as animal models for anxiety (1). Thus, the present study tested anticonflict effects of four different EOs (rose, ylang-ylang, camomile, and orange oils) in two types of conflict methods (Vogel and Geller) in order to test the hypothesis that EOs are effective for treating anxiety.

Rose, camomile, and ylang-ylang oils were selected because these oils are believed to possess antianxiety effects (18). On the other hand, it is believed that orange oil does not possess such an effect.

METHOD

Animals

Male ICR mice (Clea Japan, Tokyo) were used for analysis. The mice were 7 weeks old and weighted between 30–35 g at the start of the experiment. The mice were housed in Plexiglas cages (10 mice/cage) with stainless steel wire mesh tops and excelsior bedding. Commercial solid food (Clea Japan) was available ad lib except in Experiment 2. The animals were housed in a room artificially illuminated by fluorescent lamps on a 12 L: 12 D schedule (light period: 0700–1900 h), and the room was maintained at 25 ± 1°C.

All experiments in this study were performed in accor-

dance with the Ethics Committee for Experimental Animals of the National Institute for Environmental Studies, Japan.

Chemicals

The drugs used in this study were diazepam (DZ) (benzodiazepine agonist; Cercine Inj.®, Takeda Chem. Ind., Osaka), and flumazenil (FL) (Ro15-1788, benzodiazepine antagonist; Anexate Inj.®, Yamanouchi Pharmaceutical Co., Tokyo). Oils of rose, ylang-ylang, and camomile were extracted from flowers of *Rosa sp*., *Cananga odorata*, and *Anthenis nobilis* (or *Matricaria chamomilla*), respectively. The orange oil was extracted from the rind of *Citrus sp*. (16). All of the samples were produced by Maggie Tisserand Ltd. (Brighton, UK). DZ was diluted in 10% propylene glycol (PG) solution, and FL was diluted in physiological saline (0.9% NaCl solution). All EOs were diluted with olive oil (Wako Pure Chemical Ind., Osaka). All injection volumes were 1 ml/100 g body weight, and the injections were administered subcutaneously in the case of DZ and FL, and intraperitoneally in the case of EOs.

Experimental Procedures

Experiment 1: Vogel conflict test. A modification (19) of the method of Vogel et al. (21) was used in this study.

On the first day, individual animals that had been deprived of water for 2 days were put into separate chambers, and were allowed to drink water for 40 min (the habituation procedure). One week later, the same animals, which had again been deprived of water for 2 days prior to the test, were subjected to the Vogel conflict test 20 min after administration of DZ, EOs, or their vehicles. The mice had ad lib access to water from the spout in the chamber, and the number of licks of the spout was recorded simultaneously in each chamber for 40 min. Every 20th lick was punished by an electric shock (30 V, ca 0.1 mA, 50 Hz AC, duration = 0.3 s) through the grid, and the number of electric shocks the mice received during the 40 min was recorded.

The apparatus used in this study has been reported previously (19,20). Five Plexiglas chambers (180(W) × 100(D) × 120(H) mm) and a recorder (VC-3002-L and VC-2050-L, O'hara & Co., Tokyo) were used for the experiments. A water bottle was placed on top of each chamber, with water being available from the bottle spout, which could be reached from inside the chamber. The number of licks of the spout was counted simultaneously in each chamber. Every 20th lick was punished by an electric shock through the grid, which constituted the floor of the chamber.

Experiment 2: Geller conflict test. Animals were trained under a MULT FR20/FR20 punishment schedule of food reinforcement, which was a modified method (10) of that established by Geller and Seifter (4). The schedule consisted of four pairs of an alternating safe period of 5 min and an alarm period of 5 min. Thus, each session lasted 40 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 with 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 (about 30 lever presses/min) and a low response rate during the alarm period (about three lever presses/min). Subsequently, challenge testing sessions were conducted at intervals of 3–4 days in which DZ or EO was administrated to animals 20 min before the start of the test session. Response rates were recorded separately for the safe and alarm periods. On nonexperimental days, animals were trained without any treatment, and the stability of behavioral baseline was checked. Daily access to food was limited to maintain a food-deprivational state.

The apparatus for the Geller conflict test has been reported previously (20). The apparatus consisted of an operant chamber, a schedule controller, and a data recorder (GT-8510, GT-8005, and GT-7715, respectively; O'hara & Co., Tokyo). The chamber was made of acrylic fiber board and aluminum panels with a dimension of 80(W) × 90(D) × 100(H) mm. A stainless steel lever was set vertically in the side wall of the chamber. A saucer for food pellets was set in the same wall. The floor consisted of a stainless steel grid, wired to pass an electric current in accordance with a conflict schedule. A speaker for presenting a warning stimulus was set in the center ceiling of the chamber.

Experiment 3: Interaction between DZ or rose oil and the benzodiazepine antagonist, flumazenil (Ro15-1788) (FL) in the Vogel conflict test. FL is a benzodiazepine antagonist of benzodiazepines for the $GABA_A$ receptor complex (15,17). In this experiment, FL was first coadministrated with DZ in animals undergoing the Vogel conflict test to confirm the DZ antagonizing effect of FL in these experimental conditions. FL was then coadministrated with rose oil to animals undergoing this conflict test to examine whether rose oil acts as a benzodiazepine agonist.

Statistical Analyses

Overall differences in the means of all treatments in Experiments 1 and 2 were examined by one-way ANOVA followed by comparisons between the control and each treatment by Dunnet's test (two tailed). Results of Experiment 3 were examined by one-way ANOVA followed by Tukey's test for all combinations. A 5% of level of significance was used (22).

RESULTS

Experiment 1. Vogel Conflict Test

As expected, the positive-control drug DZ, produced a significant anticonflict effect, in that the number of electric shocks the mice received increased significantly after administration of the benzodiazepine anxiolytic (Fig. 1a), $F(4, 159) = 2.667$, $p < 0.05$.

Rose oil showed a similar anticonflict effect at 400 and 800 mg/kg (Fig. 1b), $F(4, 84) = 5.460$, $p < 0.01$. Ylang-ylang oil also tended to increase the number of electric shocks the mice received (Fig. 1c). However, the difference was not statistically significant, $F(4, 80) = 2.267$, NS. Camomile and orange oils did not show any evidence of an anticonflict effect (Fig. 1d and e). At their highest dose levels, these two EOs actually reduced the number of electric shocks the mice received, suggesting that they inhibited drinking behavior at these doses [camomile oil: $F(4, 94) = 4.204$, $p < 0.01$; orange oil: $F(5, 108) = 4.957$, $p < 0.01$]. When the data for orange oil at 1600 mg/kg is eliminated from the statistical analysis, ANOVA revealed it showed no significant effect on the target behavior, $F(4, 94) = 1.336$, $p > 0.05$, although it appeared to increase in groups administered 400–800 mg/kg.

Experiment 2. Geller Conflict Test

In this paradigm as well, the positive-control drug increased response rates during the alarm period. DZ showed

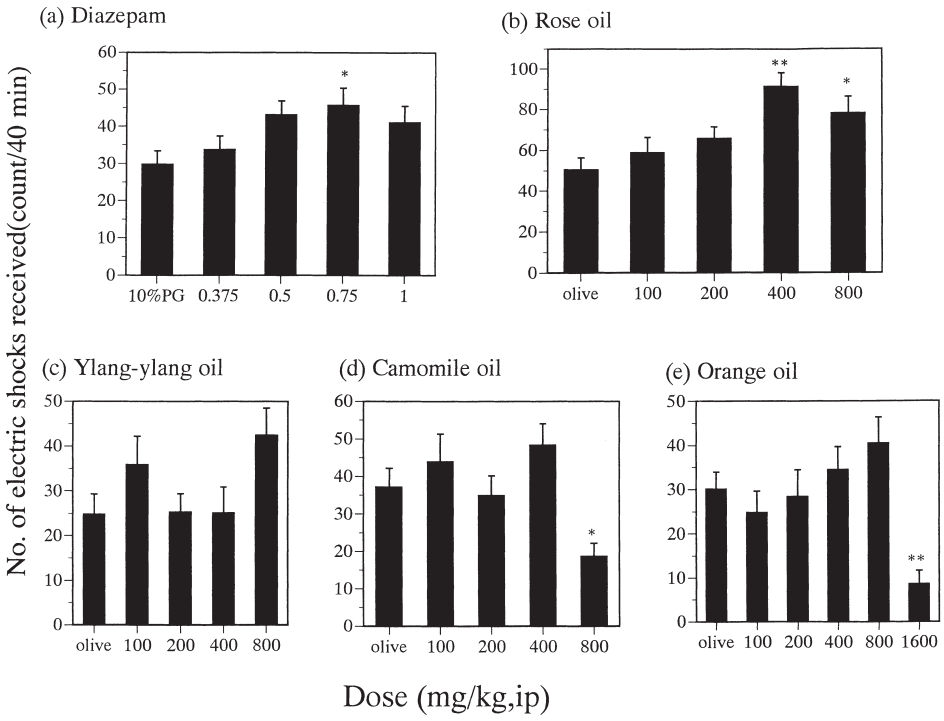

FIG. 1. Effects of (a) diazepam, (b) rose oil, (c) ylang-ylang oil, (d) camomile oil, and (e) orange oil on Vogel conflict behavior in ICR mice. Each column shows the mean value of the number of electric shocks the mice received during the 40-min session beginning 20 min after drug administration. Vertical lines denote SEM. [(a): $n = 32$–33, (b): $n = 15$–18, (c): $n = 15$–18, (d): $n = 19$–20, (e): $n = 18$–23, for each column]. Significant differences as compared with vehicle-treated control value. [$*p < 0.05$, $**p < 0.01$, respectively; Dunnet's test (two tailed)].

anticonflict effects in the conflict test, $F(4, 45) = 8.141$, $p < 0.05$ (Fig. 2a) at dose levels (1 to 2 mg/kg) that did not affect the response rate during the corresponding safe period, $F(4, 45) = 0.257$, NS.

Rose oil had a similar effect on response rates during the safe and alarm periods in that it increased response rates during the alarm period, $F(3, 36) = 2.944$, $p < 0.05$, at a dose level (400 mg/kg) that did not alter response rates in the corresponding safe period (Fig. 2b). Response rates during the safe period decreased after administration of a relatively higher dose (800 mg/kg) of rose oil, $F(3, 36) = 4.512$, $p < 0.01$. Ylang-ylang oil did not show any effects on response rates during either the safe or alarm periods throughout the range of dose levels (200–1600 mg/kg) [safe period, $F(4, 45) = 0.165$, NS; alarm period, $F(4, 45) = 0.074$, NS] (Fig. 2c). Camomile oil at dose levels of 400 and 1600 mg/kg did not affect response rates during the alarm period (Fig. 2d), $F(3, 36) = 1.936$, NS. Response rates during the safe period tended to decrease at 1600 mg camomile oil/kg, but this change was not statistically significant, $F(3, 36) = 2.455$, NS. Orange oil did not show a statistically significant effect on response rates during the alarm period, $F(4, 45) = 0.159$, NS (Fig. 2e), even at dose levels affecting response rates during the safe period, $F(4, 45) = 3.488$, $p < 0.01$.

Experiment 3. Interaction Between DZ or Rose Oil and FL as Determined in the Vogel Conflict Test

As in Experiment 1, the number of electric shocks the mice received increased at 0.75 mg DZ/kg. FL completely reversed DZ's anticonflict effects at an antagonist dose (1 mg/kg) that had no effect on this measure alone (Fig. 3a) $F(3, 113) = 2.952$, $p < 0.05$.

In contrast, FL showed no effect upon anticonflict action that rose oil exhibited, $F(3, 86) = 7.297$, $p < 0.01$, in this paradigm (Fig. 3b).

DISCUSSION

The use of EOs in the treatment of various illnesses over the centuries suggests that these EOs might have some degree of efficacy. The odor of EOs is believed to be important for their effectiveness in treating various illnesses. In fact, the odor of a given EO can change mood. However, I think that it is unlikely that the odor of EOS is potent enough to treat an illnesses. Therefore, the author hypothesized that EOs exert a pharmacological action. The present study was conducted to test this hypothesis. As EOs were administered intraperitoneally, their effects do not come from their odor. EOs are lipophilic, and were diluted with olive oil. It was highly probable that the absorption rate of EOs from olive oil to the blood stream might be quite low, and thus IP injection was selected as the administration route. In general, absorption rate is higher for IP injections than for SC injections, given that the density of blood vessels is much higher in the abdomen than under the skin.

The specific hypothesis to test for such pharmacological actions was guided by reports that many EOs may be effective in the treatment of mental illness, and in particular, that rose, ylang-ylang, and camomile oils might display antianxiety ef-

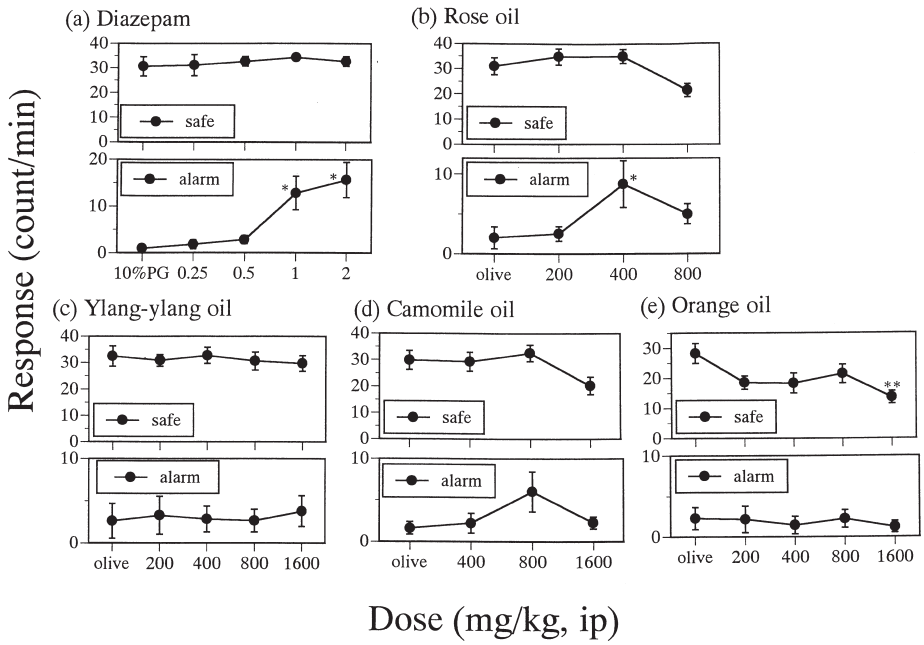

FIG. 2. Effects of (a) diazepam, (b) rose oil, (c) ylang-ylang oil, (d) camomile oil, and (e) orange oil on Geller conflict behavior in ICR mice. Upper panels of each figure show response (lever-pressing) rates during the safe (unpublished) period and lower panels show response rates during the alarm (punished) period. Points show mean values and vertical lines indicate SEM [(a): $n = 10$, (b): $n = 10$, (c): $n = 10$, (d): $n = 10$, (e): $n = 10$ for each point]. Significant differences compared with vehicle-treated control value ($*p < 0.05$, $**p < 0.01$, respectively; Dunnet's test (two tailed)].

fects in humans (18), and that orange oil is not effective in this regard. Therefore, the present study examined the effects of these oils on two types of conflict behaviors, which are standard methods for preclinical evaluation of putative anxiolytics (1) in mice.

The Vogel conflict test (21) has been used to evaluate anticonflict effects of drugs in animal studies involving primarily rats. In the present study, this method was applied to mice. A previous study from this laboratory showed that this test was applicable to mice in that it demonstrated an anticonflict effect for the benzodiazepine anxiolytic DZ, in this species (19). Subsequently, we reported that the organic solvents trichloroethylene and tetrachloroethylene have anticonflict effects demonstrable in mice by this method (20). The author has also confirmed an anticonflict effect for pentobarbital using this method in mice (data not shown).

The present study confirmed the anticonflict effect of DZ reported earlier in this test in ICR mice (19). These experimental conditions were then used to examine the anxiolytic actions of the four EOs above. The results indicated that of the four, only rose oil has a significant anticonflict effect like that of DZ. These results demonstrate for the first time that EOs such as rose oil may actually possess a specific pharmacological action.

To explore the generality of these results, the present study further examined the effects of these EOs in the Geller conflict test, another standard method for preclinical evaluation of putative anxiolytics (1). This method has also been applied to mice in the examination of various psychoactive drugs (7,10–13). The Geller anticonflict test has also been used to assess anticonflict effects of the organic solvents, trichloroethylene and tetrachloroethylene (20). The author in these studies has confirmed the previously observed anticonflict effect of DZ in this test (unpublished).

Response (lever-pressing) rates during the alarm period increased after administration of DZ without any effects on response rates during the corresponding safe period, indicating that DZ exhibited an anticonflict effect in the Geller conflict test in this strain of mice. Similarly, rose oil produced a significant anticonflict effect in this test, whereas the other three oils did not show any such effect, a finding is that analogous to the profile of results observed in the Vogel conflict test.

The present study demonstrated significant anticonflict effects of the EO rose oil, a finding that suggests that, like DZ, it possesses a pharmacological action against anxiety. On the other hand, ylang-ylang and camomile oils, which also have putative anxiolytic effects in humans (16,18), did not show any anticonflict effects in either of these two conflict tests. Orange oil, for which no claim of an antianxiety effect has been made, did not show anticonflict effects in this study. The possibility exist that ylang-ylang, camomile, and orange oils, which showed no effects, simply are not being absorbed into the blood stream. This possibility should be investigated in the future. However, although the claimed effectiveness of EOs may not be corroborated here, it is notable that the present study suggest that EOs actually possess pharmacological actions. These actions, rather than their odor, may account for the therapeutic use of EOs, despite the dearth of scientific examinations of their effectiveness for treating human disease. The pharmacology of EOs remains a promising topic for future research.

Rose oil exhibited the same anticonflict effects as did DZ, and as such, the present study undertook a pharmacological

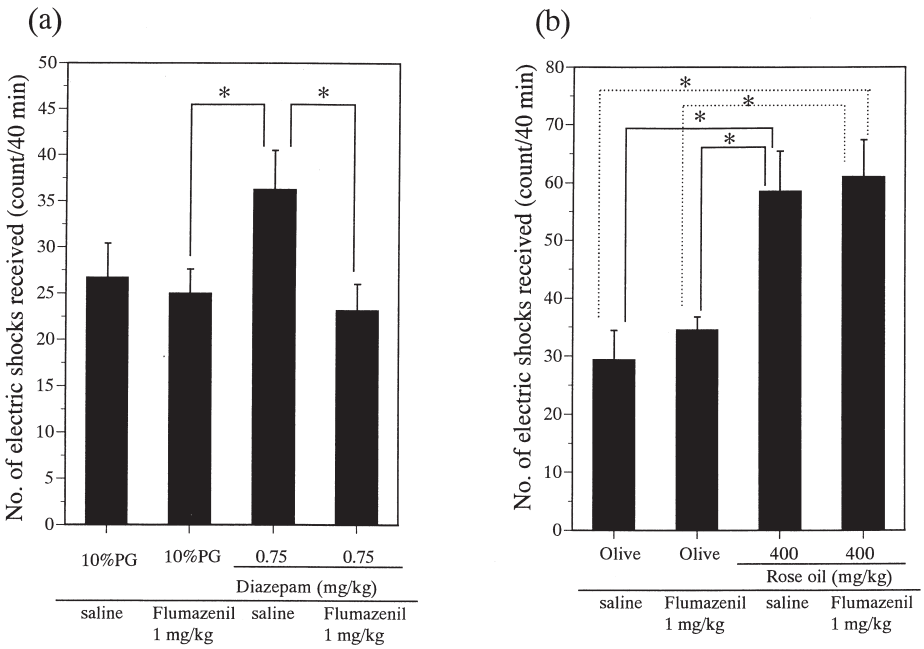

FIG. 3. Effects of (a) combined administration of 10% PG or 0.75 mg/kg of diazepam and saline or 1 mg/kg of flumazenil, and (b) combined administration of olive oil or 400 mg/kg of rose oil and saline or 1 mg/kg of flumazenil on Vogel conflict behavior in ICR mice. Each column indicates the mean value of the number of electric shocks the mice received during 40 min, and each vertical line shows SEM [(a): $n = 29$–30, (b): $n = 22$–23, for each column]. (*$p < 0.05$; Tukey's test).

analysis of rose oil to discover its mechanism of action using the probe, FL, a benzodiazepine antagonist (6). As expected, the effect of DZ was clearly antagonized by FL. On the other hand, the same dose of FL did not attenuate the anticonflict effect of rose oil, suggesting that rose oil does not share the same mechanism of action with benzodiazepine agonists, namely through benzodiazepine binding site of the $GABA_A$ receptor.

Narcotic analgesic morphine are known to cause no anticonflict effects in conflict tests in rodents (2,3,5), suggesting that the anticonflict effects of the drugs in the tests are not the product of an analgesic action. Therefore, it is highly probable that the anticonflict effect of rose oil in the present study do not result from any analgesic action of the oil, if it indeed possess an such action. The precise mechanism underlying the action of rose oil remains unclear, although it is possible to eliminate the possibilities described above. Recently, the author discovered that the σ receptor antagonist cyclazocine possesses an anticonflict action (unpublished data). In effect, it has been reported that σ receptor antagonist showed antianxiety-like effect in rodents (8). Thus, the σ receptor may be involved in the conflict behavior and may be a medium for the anticonflict action of rose oil. Alternatively, some unknown mechanism, such as signal transduction within neurons, responsible for the anticonflict action of chemicals may underlie the action of rose oil given that the mechanism for conflict behaviors have not yet been completely elucidated.

It is well known that benzodiazepines have side effects such as myorelaxation and drowsiness. The most serious side effect is drug dependency (9). Given this, nonbenzodiazepine anxiolytics have been sought, but thus far, none have been found. Selective serotonergic receptor agonists, especially $5\text{-}HT_{1A}$, were candidates for nonbenzodiazepine anxiolytics, but their antianxiety action is of a low order in comparison with that of the benzodiazepines (9). Previous studies have shown that one member of this drug class, buspirone, does not exhibit an anticonflict effect in mice (13,14). The author of the present study has also confirmed that buspirone does not show anticonflict effects in the two kinds of conflict tests used in the present study (unpublished). Rose oil behaves in the studies reported here as a nonbenzodiazepine anxiolytic. The low order of toxicity observed in this EO's wide-spread use suggests that it should be given further consideration as a candidate for a nonbenzodiazepine anxiolytic drug.

ACKNOWLEDGEMENTS

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REFERENCES

1. Commissaris, R. L.: Conflict behaviors as animal models of the study of anxiety. In: Haaren, F. V., eds. Methods in behavioral pharmacology. Amsterdam: Elsevier; 1993:443–474.
2. Cook, L.; Davidson, A. B.: Effect of behaviorally active drugs in a conflict–punishment procedure in rats. In: Garattini, S.; Mussini, E.; Randall, L. O., eds. The benzodiazepines. New York: Raven Press; 1973:321–345.
3. Furusawa, K.; Tadokoro, S.: Effects of psychoactive drugs on the conflict behavior under operant situation in Mongolian gerbils (*Meriones unguiculatus*). Jpn. J. Psychopharmacol. 10:323–329; 1990.

4. Geller, I.; Seifter, J.: The effects of meprobamate, barbiturates, *d*-amphetamine and promazine on experimentally induced conflict in the rat. Psychopharmacologia 1:482–492; 1960.
5. Goldberg, M. E.; Ciofalo, V. B.: Effects of diphenyldantoin sodium and chlordiazepoxide alone and in combination on punishment behavior. Psychopharmacologia 14:233–239; 1969.
6. Goodman, L. S.; Gilman, A., eds. The pharmacological basis of therapeutics, 8th ed. New York: Pergamon Press; 1990.
7. Haraguchi, H.; Kuribara, H.: Behavioral effects of adenosine agonists: Evaluation by punishment, discrete shuttle avoidance and activity tests in mice. Jpn. J. Pharmacol. 55:303–310; 1991.
8. Kameyama, T.; Kamei, H.; Nabeshima, T.: A role played sigma receptors in the conditioned suppression of motility in mice. Psychopharmacology (Berlin) 94:515–520; 1988.
9. Kudo, Y.; Kudo, T.: Recent progress in development of psychotropic drugs (1)—Anti-anxiety drugs. Jpn. J. Psychopharmacol. 15:75–86; 1995.
10. Kuribara, H.; Furusawa, K.; Tadokoro, S.: Effects of diazepam and methamphetamine on the conflict behavior under operant situation in mice. Asia Pacific J. Pharmacol. 1:29–31; 1986.
11. Kuribara, H.; Furusawa, K.; Tadokoro, S.: Effects of ethanol, caffeine and nicotine on conflict behavior established under an operant situation in mice. Jpn. Alcohol Drug Depend. 22:101–109; 1987.
12. Kuribara, H.; Fujiwara, S.; Yasuda, H.; Tadokoro, S.: The anti-conflict effect of MK-801, an NMDA antagonist: Investigation by punishment procedure in mice. Jpn. J. Pharmacol. 54:250–252; 1990.
13. Kuribara, H.: Comparison of the effect of 5-HT_{1A} agonist buspirone and benzodiazepine anxiolytic diazepam on conflict behavior in mice. Kitakanto Med. J. 43:289–293; 1993.
14. Kuribara, H.: Effects of SUN8399, a potent and selective 5-HT_{1A} agonist, on conflict behavior and ambulatory activity in mice: Comparison with those of buspirone, tandospirone and diazepam. Jpn. J. Pharmacol. 64:273–280; 1994.
15. Seeburg, P. H.; Wisden, W.; Verdoorn, T. A.; Prichett, D. B.; Werner, P.; Herb, A.; Lüddens, H.; Sprengel, R.; Sakmann, B.: The $GABA_A$ receptor family: Molecular and functional diversity. Cold Spring Harbor Symp. Quant. Biol. 55:29–40; 1990.
16. Seller, W.: The directory of essential oils. Essex, UK: The C. W. Daniel Company Ltd.; 1992.
17. Schoch, P.; Richads, J. G.; Haring, P.; Takacs, B.; Stabli, C.; Staehelin, T.; Haefely, W.; Mohler, H.: Co-localization of GABA receptors and benzodiazepine receptors in the brain shown by monoclonal antibodies. Nature 314:168–171; 1985.
18. Tisserand, R.: The art of aromatherapy. Essex, UK: The C. W. Daniel Company Ltd.; 1993.
19. Umezu, T.: Assessment of anxiolytics (5)—Vogel-type conflict task in mice. Jpn. J. Psychopharmacol. 15:305–314; 1995.
20. Umezu, T.; Yonemoto, J.; Soma, Y.; Miura, T.: Behavioral effects of trichloroethylene and tetrachloroethylene in mice. Pharmacol. Biochem. Behav. 58:665–671; 1997.
21. Vogel, J. R.; Beer, B.; Clody, D. E.: A simple and reliable conflict procedure for testing anti-anxiety agents. Psychopharmacologia 21:1–7; 1971.
22. Yoshimura, I.; Ohashi, Y.: Statistical analysis of toxicological data. Tokyo, Japan: Chijin-shokan; 1992.