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Anxiolytic-like effects of rose oil inhalation on the elevated plus-maze test in rats

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Abstract

The effect of rose oil inhalation (1.0%, 2.5%, and 5.0% w/w) on the elevated plus-maze (EPM) test was investigated in adult male rats and compared with the effect of diazepam (DZP) (1.0 and 2.0 mg/kg) administered intraperitoneally 30 min before testing. Exposure to rose oil produced an anxiolytic-like effect similar to DZP (anxiolytic reference drug). Thus, at some concentrations, rose oil significantly increased the number of visits to and time spent in the open arms of the EPM. Anxiolytic-like properties of rose oil were observed using the EPM, being consistent with other behavioral and clinical studies.
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Keywords: Rose oil; Elevated plus-maze; Rats; Anxiety; Diazepam; Inhalation

1. Introduction

Essential oils (EOs) are products of a generally rather complex composition comprising the volatile principles contained in various aromatic plant species that have been used in folk medicine and aromatherapy for the treatment of a variety of different illnesses, including epilepsy and anxiety (Almeida et al., 2003; Hariya et al., 2002). These characteristics have attracted the attention of many scientists to study their chemical, pharmacological, and therapeutic properties (Leal-Costa and Fonteles, 1999).

Anxiety disorders are considered the most prevalent psychiatric disease in the general population; it may be understood as the pathological counterpart of normal fear, being manifested by disturbances of mood, as well as of thought, behavior, and physiological activity (Andreatini et al., 2002; Silva and Leite, 2000).

Benzodiazepines are still the most frequently used drugs for the treatment of generalized anxiety disorder despite their undesirable side effects such as muscle relaxation, sedation, physical dependence, memory disturbance, and interaction with other drugs (Griffiths and Sannerud, 1987). Therefore, there exist a great interest in the search for new agents and other therapies (Sonavane et al., 2002).

Recent studies have shown that EOs exhibit anxiolytic effects in several behavioral models of anxiety and in human clinical trials. For instance, female patients exposed to ambient odor of orange products show low levels of anxiety, more positive mood, and high levels of calmness (Lehrner et al., 2000). Similarly, animal studies report evidence that citrus odor can restore stress-induced immunosuppression (Shibata et al., 1990) and showed antidepressant effects. Controlled studies with 14 chronic hemodialysis patients revealed that lavender and hiba oil aromas reduce depression and anxiety (Itai et al., 2000). In addition, rose and lavender oils produce significant effect in two animal conflict tests (Umezu, 2000). Specifically, the study shows that rose oil results in an alteration of the response rate during the alarm period in two conflict tests in ICR mice (Umezu et al., 2002). The effects of the identified constituents of rose oil were also examined using the same procedure as for rose oil. The results demonstrate that the 2-phenethyl alcohol and citronellol are the pharmacologically active components (Umezu et al., 2002).

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Since there are no reports about the effects of EOs on anxiety-like behavior in animals, the present preliminary investigation was undertaken to evaluate whether rose oil inhalation produces a pharmacological action similar to anxiolytic drugs administered intraperitoneally using the elevated plus-maze (EPM) method in rats.

2. Materials and methods

2.1. Animals

Adult Wistar male rats weighing 280–350 g were used in this study. All animals were housed in Plexiglas cages (five rats per cage) for at least 3 days prior to testing. Animals were maintained in controlled temperature (21 ± 2 °C) and a 12:12-h light–dark cycle (light period: 0700–1900 h). Food (Purina) and water were freely available, except during the experimental phase. All experiments in this study were performed in accordance with the Ethics Committee for Experimental Animals from Universidade Federal de São Paulo.

2.2. Chemicals

The drugs used in present study were diazepam (DZP), acquired from Roche, Brazil, and rose oil, extracted from rose flowers species and purchased from Botica Ao Veado D'Ouro, São Paulo, Brazil. DZP was diluted in 10% propylene glycol solution. Rose oil emulsion (water/oil) 1.0%, 2.5%, and 5.0% (w/w) were prepared minutes before of the experiments.

2.3. Inhalation apparatus

The inhalation apparatus consisted of four Plexiglas chambers (36 × 30 × 29 cm) built in the university. The floor was made of stainless steel grid where the animals were placed individually. The front and back walls were made of acrylic fiber containing four holes (2 cm in diameter each) where drug-embedded cotton wool was placed (2 ml per unit of respective substance—saline or rose oil). The top wall contained 30 small holes for ventilation. After two exposures, the cotton with EO was renovated to maintain the oil concentration into of apparatus.

2.4. Elevated plus-maze

The EPM apparatus was made of wood and consisted of two open arms (50 × 10 cm) opposite to one another and crossed at right angles by two enclosed arms (50 × 10 × 40 cm), with a central square of 10 × 10 cm. The floor of each open-arm was divided into three squares. The apparatus was elevated to the height of 60 cm. The maze was placed inside a sound-attenuated room, which was artificially illuminated by two 60 W white fluorescent lamp. All the parameters evaluated were recorded manually during the 5-min session. Twenty-four hours before the experiment, the animals were placed in the testing room for habituation.

2.5. Experimental procedures

Rats submitted to the inhalation treatment were placed into separated and odor-isolated chambers containing saline (control group) and different concentrations of rose oil emulsions 1.0%, 2.5%, and 5.0% w/w (experimental groups). Each rat was individually submitted to the aroma for 7 min, immediately before the tests, except for three other groups that were treated intraperitoneally in a volume of 1 ml/kg with saline (control) or DZP (1.0 or 2.0 mg/kg—standard) 30 min before being placed individually in the central square of the EPM facing a closed arm. During a period of 5 min, five measurements were taken: (1) total

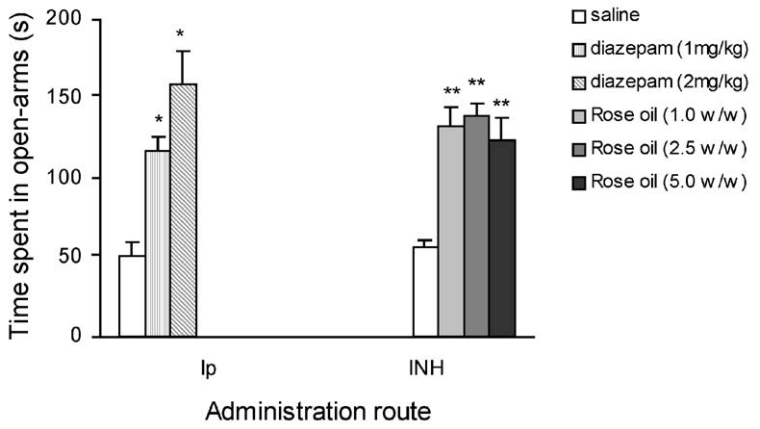

Fig. 1. Effect of different concentrations of rose oil (inhalation route) and DZP administered intraperitoneally on the time spent in open arms (s) of the EPM test in male rats. * $P < .05$ against saline-treated (Ip) animals * * $P < .05$ against saline-treated (INH) animals. Date are presented as mean ± S.D. of 10 animals per group.

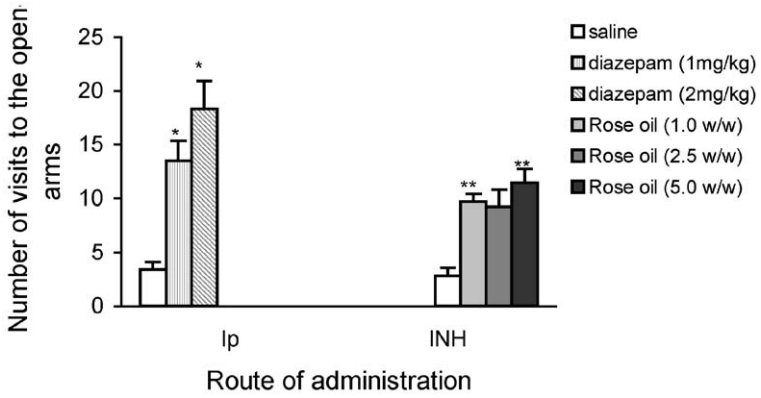

Fig. 2. Effect of different concentrations of rose oil (inhalation route) and DZP administered intraperitoneally on the number of visits to the open arms (s) of the EPM test in male rats. * $P<.05$ against saline-treated (Ip) animals. * * $P<.05$ against saline-treated (INH) animals. Data are expressed as mean ± S.D. of 10 animals per group.

number of squares traversed in the open arms (STO); (2) number of visits to the open arms (VOA); (3) number of visits to the closed arms (VCA); (4) time spent in the open arms (TOA); and (5) time spent in the closed arms (TCA).

2.6. Statistical analyses

Overall, differences in the means of all treatments were evaluated by one-way analysis of variance (ANOVA) followed by comparisons between the control and each treatment or intertreatments by Dunnet's test (two tailed). Differences were considered statistically significant when $P<.05$.

3. Results

As illustrated in Fig. 1, rose oil inhalation (1.0%, 2.5%, and 5.0% w/w) induced an increase in the time spent in the open arms [$F(3,36)=15.22$, $P<.0001$]. The data summarized in Fig. 2 revealed that the number of visits to the open arms was also significantly affected by the different rose oil concentrations [$F(3,36)=12.88$, $P<.0001$].

Fig. 3 shows a significant effect of all inhalation treatments on squares traversed in the open arms [$F(3,36)=11.17$, $P<.0001$]. According to the analysis, no significant difference was observed between the rose oil concentrations and DZP treatment.

4. Discussion

The increase in the time spent in and the number of visits to the open arms strongly indicates the anxiolytic activity of the EO treatment produced by the inhalation procedure. An increase of time spent in as well as the number of visits to the open arms, compared to the control group, provides a measurement of attenuation of the fear-induced inhibition of exploratory activity presented by anxiolytic drugs (Jones et al., 1992).

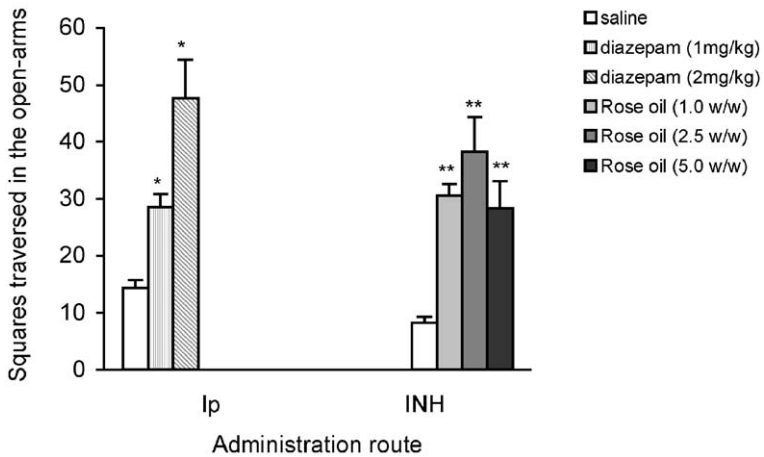

Fig. 3. Effect of different concentrations of rose oil (inhalation route) and DZP administered intraperitoneally on the number of squares traversed in the open arms (s) of the EPM test in male rats. * $P<.05$ against saline-treated (Ip) animals. * * $P<.05$ against saline-treated (INH) animals. Data are presented as mean ± S.D. of 10 animals per group.

Rose oil inhalation increased yet another parameter, STO, an effect that was also observed in the DZP-treated group, providing an additional indication of antianxiety activity. Increased locomotion in the open arms is indicative of increased dopaminergic transmission that may produce secondary changes in 5-HT levels elicited by anxiolytic drugs (Jones et al., 1992; Sonavane et al., 2002). Similar effects were described by Umezu et al. (2002) using two other animal models of anxiety: the Geller and Vogel conflict tests in which rose oil administered intraperitoneally produced an increasing effect on the response rate during the alarm period in the Geller conflict test and increased the number of electric shocks mice received in the Vogel conflict methodology. This anxiolytic-like effect was attributed to citronellol and 2-phenethyl alcohol, both isolated from essential rose oil. The EPM apparatus has been used to evaluate anxiolytic as well as anxiogenic effect of drugs. Rats and mice, under normal behavioral pattern, have more preference for the enclosed arms, which is safer and less bright (Dawson et al., 1995; Lister, 1987; Pellow et al., 1985).

Although the findings of the present systematic study have shown a preliminary trial to establish a new methodology for testing the possible anxiolytic effect of odorant preparations in the rat, it must be viewed with caution since it is suggested that the inhalant route has an immediate anxiety alleviating, but short lived, effect in patients (Cooke and Ernst, 2000; Xi et al., 1994). On the other hand, a recent and important study shows that neurons of the vomeronasal organ, which is considered an accessory olfactory system, can actually detect odorants and pheromones (Sam et al., 2001). This suggests that in mammals, as insects, odorous compounds released from plants or others animal species may act as 'semiochemicals' signaling molecules that elicit behavioral changes. Additionally, it was identified, cloned, and functionally expressed as a previously undescribed human testicular olfactory receptor, hOR17-4, which is activated by odorants, and in special presents a significant response to bourgeonal, a component of rose oil (Luo et al., 2003; Spehr et al., 2003). More investigations are necessary to evaluate the precise mechanism of anxiolytic action of natural odor in comparison to synthetic odorant compounds and adverse effect of rose oil inhalation despite its wide spread popular use.

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