Assessment of anxiolytic effect of nerolidol in mice

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Abstract:
Aim and Objectives: The present study was to assess the anxiolytic effect of nerolidol in mice.

Materials and Methods: The anxiolytic activity was examined using the elevated plus maze (EPM) and open field test (OFT), and motor coordination by rotarod test. Thirty Swiss albino mice were divided into five groups of six mice each. Group 1 received vehicle control (normal saline); Group 2 received diazepam (1 mg/kg); Groups 3, 4, and 5 received nerolidol 12.5, 25, and 50 mg/kg, respectively.

Results: Nerolidol (12.5, 25, and 50 mg/kg) significantly \((P < 0.05)\) increased the time spent and a number of entries in open arm as compared to vehicle control in EPM test. In OFT, the nerolidol showed a significant \((P < 0.05)\) increase in number of rearings and time spent in center and periphery, suggesting exploratory behavior of animals. Furthermore, nerolidol did not alter the fall down latency in rotarod test.

Conclusion: Our findings indicated that nerolidol exerts an anxiolytic effect without altering the motor coordination.

Key words:
Anxiety, diazepam, elevated plus maze, nerolidol, open field test

Anxiety is the common psychiatric disorder that affects approximately one-eighth of the world population.\(^1\) Currently, various psychotropic drugs are available to grapple with this psychiatric disorder. Among the available psychotropic drugs, benzodiazepines are commonly prescribed to the patients. However, the regular use of the benzodiazepines leads to various side effects.\(^2\) Thus, there is a need to explore newer and safer pharmacological agents to treat anxiety. To resolve this issue, various alternative therapies such as aromatherapy have been used to manage the anxiety and other psychiatric disorder.

Nerolidol is a sesquiterpene alcohol frequently found as a major component of essential oil obtained from various species of well-known aromatic plants such as Afframum pruinatum (<90%), Myrcangia cucullata (<90%), Siparuna guianensis (90%), Melaleuca quinquenervia (87%), Piper clausenianum (83%), Eucalyptus nova-anglica (<80%), Salvia runcinata (72%), and many more. It is widely used as a fragrance ingredient in the various pharmaceutical formulations.\(^3\)

Studies focusing on nerolidol have shown various pharmacological properties such as antiulcer,\(^4\) antioxidant,\(^5\) antifungal,\(^6\) anti-inflammatory,\(^7\) and anticonvulsant.\(^8\) However, the literature revealed that nerolidol has been least explored with respect to central nervous system. Therefore, this prompted us to investigate the anxiolytic effect of nerolidol in different experimental models.

Materials and Methods

Animals
Male Swiss laka mice, weighing 20–30 g obtained from Central Research Institute, Kasauli, were employed in the present study, in different groups \((n = 6)\). The animals were housed in standard cages and were maintained at room temperature with natural day and night cycles. The animals were allowed free access to food (standard laboratory rodents chow) and water during the study period. All experiments were carried out between 07:00 and 16:00 h. All procedures were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The experimental protocol was approved by the Institutional Animal Ethical Committee established by the University (CPCSEA Protocol No: 107/99/CPCSEA/2014-13).

How to cite this article: Goel RK, Kaur D, Pahwa P. Assessment of anxiolytic effect of nerolidol in mice. Indian J Pharmaco 2016;48:450-2.
Drugs and Treatments
Nerolidol (Sigma-Aldrich) was diluted with tween eighty to prepare nerolidol emulsions (12.5%, 25%, and 50%) before the experiments. Diazepam (Jackson Laboratories Pvt. Ltd., India) was dissolved in normal saline and used as positive control (1 mg/kg). It was administered intraperitoneally (i.p.) at a volume of 0.1 ml/10 g of body weight.

Effect of Nerolidol on Behavior of Mice in Elevated Plus Maze
This is a behavioral paradigm that takes advantage of the conflict behavior of rodents between exploration of a novel area and aversion to open and elevated spaces.[9] The maze consists of four arms with opposite facing two open (16 cm × 5 cm) and two enclosed arms (16 cm × 5 cm × 12 cm) connected by a central platform. The whole maze is raised 25 cm above the floor. Mice were tested on the plus maze in a room with low direct lighting and low noise levels. On the day of testing, the mouse was placed at the center of the maze with head facing an open arm and allowed to explore for 5 min. The number of entries and time spent in each arm was recorded. The entry into arm was considered when all four paws of the animal were placed on the arm. The maze was wiped clean with 70% ethanol solution and dried after testing each mouse. Increase in time spent and frequency of open arms entries relative to control mice were considered as indicators of anxiolytic behavior.

Open Field Test
Open field test (OFT) was used to evaluate locomotor and thigmotactic behavior of mice.[9] The open field consisted of 72 cm × 72 cm plexiglass square with 36 cm walls. For analysis, the chamber was divided into sixteen 18 cm × 18 cm squares. A central square was withdrawn in middle of the open field with red line for counting the crossings in case of high locomotor activity. Each mouse was placed in the center of the open field area and allowed to explore it for 5 min. During the 5 min test session, the variables of locomotor activity (number of black line crossings and central square entries), basic movements, fine movements (such as head-twitching and grooming), and time spent in periphery and central zones were recorded. Open field area was cleaned with 70% ethanol solution and let dry after testing each mouse.

Rotarod Test
In this test, the animals were preselected in a training session 24 h before the test based on their ability to remain on the bar (at 12 rpm) for 2 min. Groups of preselected animals were treated with vehicle, nerolidol (12.5, 25, and 50 mg/kg; i.p.), or diazepam (1 mg/kg; i.p.). Thirty minutes after the treatment, the animals were placed with all four paws onto the bar, and the fall down latency was evaluated.[10]

Results
Effect of Nerolidol on Behavior of Mice in Elevated Plus Maze
In the elevated plus maze (EPM), diazepam-treated animals showed ($P < 0.001$) a significant increase in a number of entries and time spent in the open arm as compared to vehicle control animals. Similarly, nerolidol-treated animals (12.5, 25, and 50 mg/kg) exhibited significant increase ($P < 0.05$) in the number of entries and time spent in the open arm as compared to vehicle control animals. However, nerolidol-treated animals showed a significant reduction ($P < 0.05$) in the time spent in enclosed arm as compared to vehicle control animals [Table 1].

Discussion
Essential oils are concentrated volatile oils mainly found in aromatic plants and characterized by their fragrance. They have been widely used in the aromatherapy due to their medicinal properties. Moreover, various essential oils have been reported to possess anxiolytic effects through animal model of anxiety.[11,12] In the present study, nerolidol an essential oil significantly decreased the levels of anxiety. The methodology used to evaluate anxiolytic effect is discussed below.

Among models used to evaluate anxiolytic effect, the EPM is considered to be most widely validated test because it uses natural stimuli such as brightly lit open space, the fear of a new, and fear of balancing on a relatively narrow raised platform. In the EPM, an anxiolytic drug specifically increases the number of entries and the time spent in the open arms.[13,14] Similarly, in the current study, nerolidol-treated animals significantly increased the number of entries and the time spent in the open arms indicating its anxiolytic effect. Diazepam is a known anxiolytic drug used in the current study also increased the number of entries and the time spent in the open arms as expected.

In the OFT, anxiolytic treatment decreases the anxiety-induced inhibition of exploration.[5,15] Nerolidol 50 mg/kg dose showed almost similar exploratory behavior as that of standard anxiolytic drug used in the current study.

A deficit in motor coordination would like to affect the performance in the behavioral tests. Therefore, we evaluated the motor effects of nerolidol in the rotarod test, a conventional animal model used to evaluate the motor coordination.[16] Our results showed that anxiolytic dose of nerolidol (12.5, 25, and 50 mg/kg) as well as diazepam (1 mg/kg) had no significant effect on motor coordination.

The nerolidol has been reported as a positive modulator of gamma aminobutyric acid receptor.[16,17] Moreover, anxiolytic profile and effect on motor coordination at anxiolytic dose of nerolidol is similar to that of diazepam, suggesting GABAergic modulation as a possible mechanism for its anxiolytic effect. However, further experiments are required to ascertain this.

Conclusion
Nerolidol showed the anxiolytic effect without altering the motor coordination in mice. Further pharmacological investigations are warranted to elucidate the exact mechanism of action.
Table 1: Effect of nerolidol on behavior of mice in elevated plus maze

| Treatment       | Time spent in the open arm (s) | Time spent in the enclosed arm (s) | Entries into open arm | Entries into enclosed arm |
|-----------------|---------------------------------|------------------------------------|-----------------------|---------------------------|
| Vehicle-control | 85.33±6.72                      | 177.83±8.44                        | 8±1.15                | 8.5±1.56                 |
| Diazepam        | 196.66±7.6***                   | 55.33±4.97***                      | 17±1.23***            | 4±0.36**                 |
| Nerolidol 12.5  | 136.5±6.81***                   | 100.66±10.17***                    | 11±0.77*              | 7.5±0.42                 |
| Nerolidol 25    | 154.66±9.18***                  | 76.33±7.96***                      | 11±0.77               | 5.33±0.61*               |
| Nerolidol 50    | 169.66±7.12***                  | 64.66±6.43***                      | 13.66±0.55**          | 4.33±0.33**              |

Values are represented as means±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001; as compared to vehicle control animals (one-way ANOVA followed by Dunnett’s test). Nerolidol 12.5, 25, and 50: Nerolidol 12.5, 25 and 50 mg/kg. SEM=Standard error of mean

Table 2: Effect of nerolidol on behavior of mice in open field test

| Treatment       | Number of rearing | Time spent in the center (s) | Time spent in the periphery (s) |
|-----------------|-------------------|------------------------------|---------------------------------|
| Vehicle-control | 10±0.78           | 26.5±4.5                     | 253.66±16.48                   |
| Diazepam        | 17.8±1.19***      | 83.5±3.14***                 | 84±6.51***                     |
| Nerolidol 12.5  | 13.5±2.01***      | 50±3.65***                   | 126.5±12.68***                |
| Nerolidol 25    | 15±1.83***        | 64±3.18***                   | 111.66±8.97***                |
| Nerolidol 50    | 21.3±3.26***      | 74±3.21***                   | 95.33±5.94***                 |

Values are represented as means±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001; as compared to vehicle control animals (one-way ANOVA followed by Dunnett’s test). Nerolidol 12.5, 25 and 50: Nerolidol 12.5, 25 and 50 mg/kg. SEM=Standard error of mean

Table 3: Effect of nerolidol on the rotarod test

| Treatment       | Fall down latency (s) |
|-----------------|-----------------------|
| Vehicle-control | 298.33±0.84           |
| Diazepam        | 293±1.59              |
| Nerolidol 12.5  | 297.66±0.91           |
| Nerolidol 25    | 295.33±1.64           |
| Nerolidol 50    | 292.66±2.34           |

Values are represented means±SEM (n=6). Nerolidol 12.5, 25, and 50: Nerolidol 12.5, 25 and 50 mg/kg. SEM=Standard error of mean

Acknowledgment
The authors are deeply grateful to the Council of Scientific and Industrial Research (CSIR), Pusa, New Delhi, India, for providing financial assistance (Vide F.No. 38 (1339)/12/EMR-II and Department of Pharmaceutical Science and Drug Research, Punjab University, Patiala to provide infrastructures and other facilities to carry out the research work.

Financial Support and Sponsorship
The authors are deeply grateful to the Council of Scientific and Industrial Research (CSIR), Pusa, New Delhi, India, for providing financial assistance (Vide F.No. 38 (1339)/12/EMR-II) for the current study.

Conflicts of Interest
There are no conflicts of interest.

References
1. Mesfin M, Asres K, Shibeshi W. Evaluation of anxiolytic activity of the essential oil of the aerial part of Foeniculum vulgare Miller in mice. BMC Complement Altern Med 2014;14:310.
2. Thippeswamy BS, Mishra B, Veerapur VP, Gupta G. Anxiolytic activity of Nymphaea alba Linn. in mice as experimental models of anxiety. Indian J Pharmacol 2011;43:50-5.
3. Nogueira Neto JD, de Almeida AA, da Silva Oliveira J, Do Santos PS, de Sousa DP, de Freitas RM. Antioxidant effects of nerolidol in mice hippocampus after open field test. Neurochem Res 2013;38:1861-70.
4. Kloppel FC, Lemos M, Sousa JP, Comunello E, Maestro EL, Bastos JK, et al. Nerolidol, an anxiolitic constituent from the essential oil of Baccharis dracunculifolia DC (Asteraceae). Z Naturforsch C 2007;62:537-42.
5. Park MJ, Gwas KS, Yang I, Kwak WS, Jeung EB, Chang JW, et al. Effect of citral, eugenol, nerolidol and alpha-terpineol on the ultrastructural changes of Tricholobion montaguiophyes. Fitoterapia 2009;80:290-6.
6. Tung YT, Chua MT, Wang SY, Chang ST. Anti-inflammation activities of essential oil and its constituents from indigenous cinnamon (Cinnamomum osmophloeum) twigs. Bioresour Technol 2008;99:3908-13.
7. Baxendale S, Holdsworth CJ, Meza Santocoy PL, Harrison MR, Fox J, Parkin CA, et al. Identification of compounds with anti-convulsant properties in a zebrafish model of epileptic seizures. Dis Model Mech 2012;5:773-84.
8. Pellow S, Chopin P, File SE, Briley M. Validation of open: Closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149-67.
9. Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. Eur J Pharmacol 2003;463:3-33.
10. Dunham NW, Miya TS. A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc Am Pharm Assoc 1957;46:208-9.
11. Cheng BH, Sheen LY, Chang ST. Evaluation of anxiolytic potency of essential oil of S(+)-linalool from Cinnamomum osmophloeum ct. linalool leaves in mice. J Tradit Complement Med 2014;5:27-34.
12. Souto-Maior FN, de Carvalho FL, de Morais LC, Netto SM, de Sousa DP, de Almeida RN. Anxiolytic-like effects of inhaled linalool oxide in experimental mouse anxiety models. Pharmaco Biochem Behav 2011;100:259-63.
13. Nic Dhonchnadhia BA, Bourin M, Hascoet M. Anxiolytic-like effects of 5-HT2 ligands on three mouse models of anxiety. Behav Brain Res 2003;140:203-14.
14. Yadav AV, Kawale LA, Nade VS. Effect of Morus alba L. (mulberry) leaves on anxiety in mice. Indian J Pharmacol 2008;40:52-6.
15. Nagaraja TS, Mahmood R, Krishna V, Thippeswamy BS, Veerapur VP. Evaluation of anxiolytic effect of Erythrina myrsinifolia Gamb. in mice. Indian J Pharmacol 2012;44:489-92.
16. Fonseca DV, Salgado PR, de Carvalho FL, Salvadori MG, Penha AR, Leite FC, et al. Nerolidol exhibits antinociceptive and anti-inflammatory activity: Involvement of the GABAergic system and proinflammatory cytokines. Fundam Clin Pharmacol 2016;30:14-22.
17. Heldwein CG, Silva LL, Reckziegel P, Barros FM, Bünger ME, Baldisserotto B, et al. Participation of the GABAergic system in the anesthetic effect of Lippia alba (Mill.) N.E. Brown essential oil. Braz J Med Biol Res 2012;45:436-43.