Aim: Study of Anxiolytic Activity Using Elevated Plus Maze Method

References:

1. Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of Neuroscience Methods, 14(3), 149-167.

2. Vogel, H. G. (2008). Drug Discovery and Evaluation: Pharmacological Assays. Springer.

Introduction:

The Elevated Plus Maze (EPM) is a widely used behavioral assay to assess anxiolytic (anxiety-reducing) activity in rodents. The EPM consists of two open arms and two closed arms elevated above the ground. Rodents typically avoid the open arms due to their aversion to open spaces. Anxiolytic compounds increase the time spent and entries into the open arms.

Objective:

To determine the anxiolytic activity of a test compound using the elevated plus maze method in mice.

Materials and Reagents:

- Mice (20-25 g, either sex)
- Test compound
- Standard anxiolytic (e.g., diazepam)
- Saline or vehicle (control)
- Elevated Plus Maze apparatus
- Stopwatch or timer
- Animal cages
- Disposable gloves
- Laboratory coat

Procedure:

Animal Preparation

1. Acclimatize the mice to the laboratory conditions for at least one week before the experiment.

2. Fast the mice overnight with free access to water prior to the experiment.

Experimental Groups:

Divide the mice into the following groups, with a minimum of six animals per group:

1. Control group: Receive saline or vehicle

2. Standard group: Receive a standard anxiolytic (e.g., diazepam)

3. Test groups: Receive different doses of the test compound

Administration of Compounds:

1. Administer the test compound, standard anxiolytic, or vehicle intraperitoneally (i.p.) according to the group designation.

2. Allow 30 minutes for absorption.

Elevated Plus Maze Testing:

1. Place the mouse in the center of the EPM facing one of the open arms.

2. Start the stopwatch and record the following parameters for 5 minutes:

- Number of entries into open arms
- Number of entries into closed arms
- Time spent in open arms
- Time spent in closed arms

3. An entry is defined as all four paws of the mouse entering an arm.

Calculation of Anxiolytic Activity:

1. Calculate the mean number of entries and time spent in open and closed arms for each group.

2. Compare the values for the test and standard groups with the control group.

3. An increase in the number of entries and time spent in the open arms indicates anxiolytic activity.

Results and Discussion:

1. Present the data in a table showing the number of entries and time spent in open and closed arms for each mouse in all groups.

2. Calculate and present the mean number of entries and time spent in open and closed arms for each group along with the standard deviation.

3. Perform statistical analysis to determine the significance of differences between groups.

4. Discuss the results, comparing the anxiolytic activity of the test compound with the control and standard groups. A significant increase in the number of entries and time spent in open arms compared to the control group indicates anxiolytic activity.

Safety and Ethical Considerations:

1. Ensure all experimental procedures involving animals comply with institutional and national ethical guidelines for the care and use of laboratory animals.

2. Handle all animals with care and minimize their distress.

3. Dispose of all biological waste according to safety guidelines.

Conclusion:

Summarize the findings, stating whether the test compound demonstrated significant anxiolytic activity and how it compared to the standard anxiolytic.

Data Table

Group	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6	Mean Number of Entries (Open Arms) ± SD	Mean Time Spent (Open Arms) ± SD (seconds)
Control	2	3	2	3	2	2	2.33 ± 0.52	45.0 ± 5.10
Standard (Diazepam)	6	7	6	8	7	6	6.67 ± 0.82	150.0 ± 8.37
Test (10 mg/kg)	4	5	4	5	4	4	4.33 ± 0.52	90.0 ± 6.32