

AIM :

To study the anxiolytic (anti-anxiety) effect of diazepam in mice using elevated plus- maze apparatus.

REQUIREMENTS :

Animal : Mice (20-25 g)

Solutions : Diazepam (Dose 2 mg/kg ip. Prepare a stock solution containing 0.2 mg/ml of the drug and inject 1 ml/100g of body weight of the mouse).

Equipment : Plus-maze consisted of two open arms (16 x 5 cm) and two enclosed arms (16 x 5 x 12 cm) with an open roof and is elevated to a height of 25 cm, stop watch.

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PRINCIPLE :

- Elevated plus-maze is the most simple apparatus to study anxiety response and the effect of almost all type of anti-anxiety agents.
- Exposure of the animal to novel maze alley evokes an approach-avoidance conflict which is stronger in open arm as compared to enclosed arm.
- Rodents (rats and mice) have aversion for high and open space and prefer enclosed arm and, therefore, spend greater amount of time in enclosed arm.

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- When animals enter open arm, they freeze, become immobile, defecate and show fear like movements.
- The plasma cortisol level is also reported to be increased, as a true reflection of anxiety.
- Major advantages of this test procedure are :
 - (a) it is simple, fast and less time consuming
 - (b) no prior training or noxious stimuli (sound or light) is required
 - (c) it is predictable and reliable procedure for studying anxiety response as well as anti-anxiety action of drugs

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1. Weigh and number the mice.

2. Divide them into two groups, each comprising of at least 6 mice for control and drug (diazepam) treatment.

3. Place the animal individually in the centre of the maze, head facing forwards open arm and start the stop watch and note following parameters for five minutes:

a) First preference of mouse to open or enclosed arm.

b) Number of entries in open and enclosed arms (An arm entry defined as the entry of four paws into the arm).

c) Average time each animal spends in each arm (Average time = total duration in the arm/number of entries).

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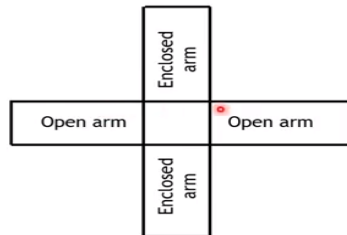
4. Inject diazepam to the test group.

5. After 30 min place the animal individually in the centre of the maze and note all parameters as described under step 3.

6. Compare the preference of the animal to open/enclosed arm, average time spend in open arm and number of entries in open arm in each group.

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OBSERVATIONS :

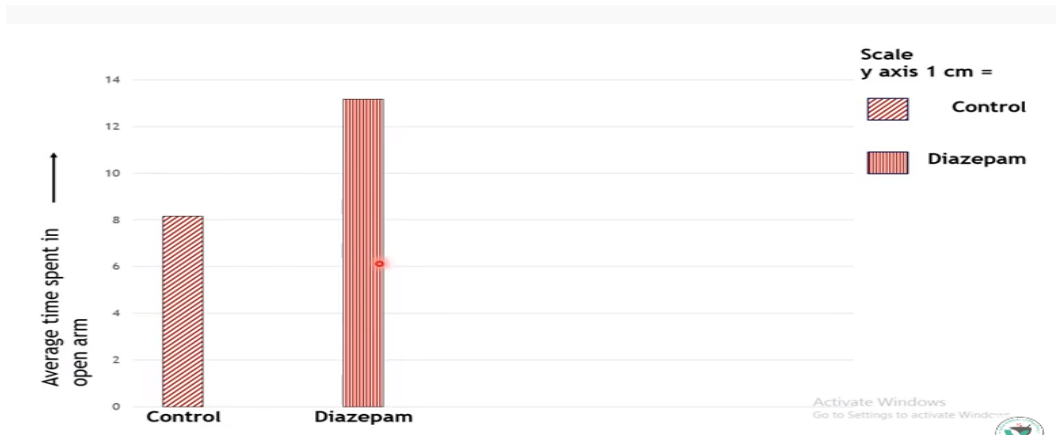
Sr. No	Body weight (g)	Treatment	% Preference to open arm	Open arm	
				No. of entries	Average time spent
1	20	Control	50	5	7
2	22		45	6	8
3	22		42	5	7
4	21		48	7	9
5	20		45	6	10
6	22		40	6	8
Mean				5.83	8.17
1	20	Diazepam	80	8	12
2	23		88	7	13
3	22		85	9	13
4	21		86	8	14
5	20		82	9	15
6	22		90	9	12
Mean				8.33	13.17

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GRAPH :

Plot a graph of

y axis : Average time spent in open arm in control and treatment group



INFERENCE :

Diazepam increases per cent preference of the animal to open arm and increase number of entries and average time spent by the mouse in the open arm. Thus Diazepam has antianxiety activity.

REFERENCE

S. K. Kulkarni; Handbook of Experimental Pharmacology; 4th Edition; Vallabh Prakashan; 146 - 148.



Plus Maze apparatus