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Original Research Article

Anxiolytic Effect of Zinc Oxide in Wistar Albino Rats: An Elevated Plus-Maze Study

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Abstract

Introduction: Anxiety disorders are prevalent psychiatric illnesses, and the search for effective treatments with minimal side effects continues. Zinc, an essential micro-nutrient, plays a crucial role in central nervous system physiology and has been implicated in neuropsychiatric disorders.

Methods: This study evaluated the anxiolytic activity of zinc oxide in albino rats using the elevated plus-maze model. Rats were divided into seven groups and administered zinc oxide (7.5, 15, 30 mg/kg) or diazepam (1, 2 mg/kg) intraperitoneally. Parameters recorded included time spent in open and closed arms and number of entries into each arm.

Results: Zinc oxide (15 mg/kg) significantly increased time spent in open arms (113.33±3.6 sec) and entries into open arms (3.55±0.23) compared to controls. Diazepam (2 mg/kg) also showed significant anxiolytic effects. The combination of zinc oxide (7.5 mg/kg) and diazepam (1 mg/kg) produced antianxiety activity, indicating potentiation.

Conclusion: This study demonstrates the anxiolytic effects of zinc oxide (15 mg/kg) and diazepam (2 mg/kg) in albino rats using the elevated plus-maze model. Zinc oxide's mechanism of action may involve inhibition of post-synaptic NMDA receptors. The potentiation of diazepam's effect by low-dose zinc oxide suggests a potential therapeutic combination for anxiety disorders. Further studies are necessary to confirm these findings and explore zinc's role in treating central nervous system disorders.

Keywords: Anxiolytic Effect, Zinc Oxide, Elevated Plus-Maze.

Introduction

Psychiatric disorders are usually associated with significant distress or disability in social, occupational or other important activities. [1] Anxiety disorders are the most prevalent psychiatric illnesses in the general community and are present in 15–20% of outpatient departments. [2] The average age of onset of generalized anxiety disorder is around 16 years and it has considerably increased in recent years. [3]

Anxiety is diffuse, unpleasant and highly vague feeling of apprehension with one or more bodily symptoms. Anxiety is considered excessive or pathological when it arises in absence of stress or challenge, when it is out of proportion to the challenge or stress in severity or duration, when it results in significant distress and when it results in psychological, social, occupational, biological and other impairment. [4]

The primary treatments for anxiety-related disorders include the benzodiazepines, the azapirone buspirone, and beta adrenergic antagonists. The benzodiazepines are effective anxiolytics in both acute and chronic conditions. There is concern regarding their use because of their potential for dependence and abuse as well as negative effects on cognition and memory. [5] Buspirone takes several weeks to show effects and requires thrice daily dosing. Beta-adrenergic antagonists, particularly those with higher lipophilicity like propranolol are occasionally used for performance anxiety such as fear of public speaking but their use is limited due to significant side effects such as hypotension. [6, 8] Hence, the search for more effective drugs with fewer side effects is on.

Zinc is an essential micro-nutrient. It plays an important role in central nervous system

physiology. The presence of zinc has been confirmed in neocortex, hippocampus and amygdala. [7] Studies suggest the role of zinc in pathogenesis of neuropsychiatric disorders, such as epilepsy, mood disorders and neurodegenerative diseases. According to the study done by Takeda et al. neuropsychological behavior such as anxiety and aggression is increased in zinc-deficient rats and mice. Rats fed on a zinc-deficient diet for a period of 2 weeks also exhibit anxiety-like behaviour which was shown by Tassabehji et al. [7]

Based on the present review, the study was undertaken with the purpose of evaluating the anti-anxiety activity of zinc oxide in animal model of anxiety and its comparison to Diazepam a standard drug. The study also aims at identifying the ability of zinc oxide to potentiate the anxiolytic effect of Diazepam.

Objectives

1. To evaluate the anxiolytic activity of zinc oxide alone in albino rats.
2. To compare the anxiolytic activity of zinc oxide with standard drug Diazepam in albino rats.

Materials and Methods [9]

Healthy albino rats of either sex weighing between 150 to 250 g were used. These animals were housed in the central animal house, procured from National Institute of Nutrition, Hyderabad. The animals were housed under standard laboratory conditions, maintained on 12:12 light dark cycle and had free access to food and water. The animals were acclimatized to laboratory conditions 1 week before the test. All the experiments were performed between 10 a.m. and 4 p.m. at room temperature in noiseless, well-illuminated room. The animals were allowed food and water ad-libitum during the experiment. The animals were allowed food and water ad-libitum during the experiment. The study was done after approval from Institutional Animal Ethics Committee (IAEC).

Drugs and Chemicals

1. Diazepam (Inj. Calmpose, Ranbaxy lab, available in strength of 5 mg/ml.)
2. Zinc oxide powder (S Dfine Chemical Laboratory.)

The drugs were dissolved in normal saline and solutions were prepared .The solutions were prepared freshly before the experiments and concentrations were so adjusted that the volume of injection will be 0.1 ml per 100 gm of body weight. All the drugs were injected intraperitoneally, under aseptic precautions. The injections were given using 1ml syringe which is divided into 100 units. Zinc oxide was given at the dose of 7.5, 15, 30 mg/kg body weight and diazepam at the dose of 1,2 mg/kg body weight.[10] Rats were selected by the process of randomization and were placed into 7 different groups each having 6 rats

Table 1: Grouping of Rats: Elevated Plus Maze Model. (N = 42)

Group No.	Groups (n=6)	Doses mg/kg body.wt	Route
1.	Control (NS)	0.5ml/rat	i.p
2.	Diazepam (standard)	1	i.p
3.	Diazepam (standard)	2	i.p
4.	Zinc oxide (test drug)	7.5	i.p
5.	Zinc oxide (test drug)	15	i.p
6.	Zinc oxide (test drug)	30	i.p
7.	Zinc oxide + Diazepam	7.5 + 1	i.p

(i.p – intraperitoneal)

Elevated plus maze model :

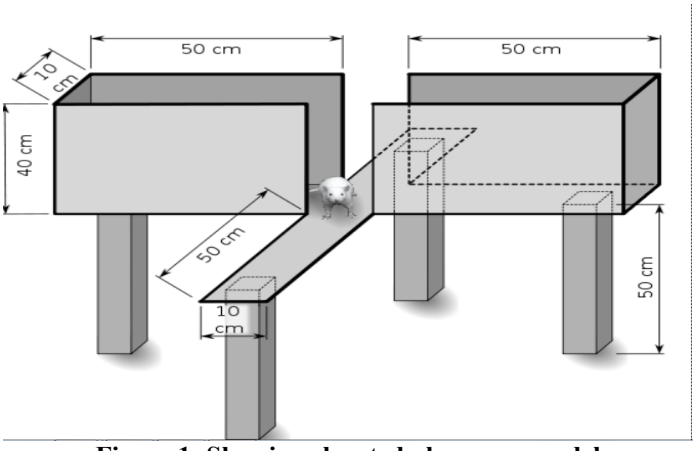

Figure 1: Showing elevated plus maze model

Description of apparatus: Elevated plus maze apparatus consists of 2 open and 2 closed arms with dimensions 50 x 10 cm and 50 x 10 x 40 cm respectively. It has an open roof. Entire maze is elevated 50 cm from floor.

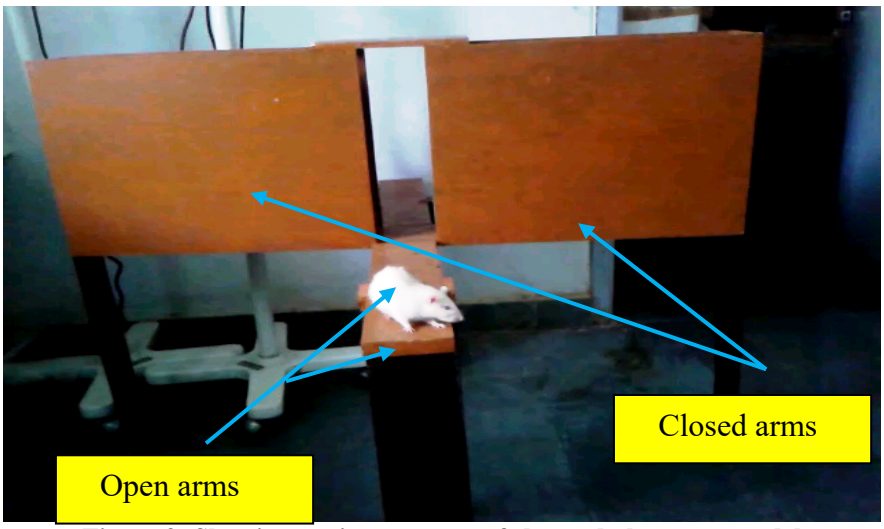

Figure 2: Showing rat in open arm of elevated plus maze model

Elevated plus maze

Procedure: Initially drug was given i.p. and after 30 min, rat was placed individually at the center of maze with head facing towards an open arm and allowed to move for 5 min (300 sec). In animals belonging to Group VII (combination), zinc oxide was administered initially, followed by Diazepam after 30 min. i.e. parameters were noted 1 hr after zinc oxide administration.

The parameters recorded were

- Time spent in open arm,
- Time spent in closed arm,
- Number of entries into open arms and
- Number of entries into closed arms.

Normally, rats (rodents) prefer to be in closed arms, but after anxiolytic drug administration they prefer more time in open arms.

Statistical analysis: Statistical Analysis is done by SPSS software. Data is analyzed by using Analysis of Variance (ANOVA) with drug treatment as independent factor. p value < 0.05 is considered as statistically significant. Post-hoc comparisons are made using Least Significant Difference (LSD) test.

Observation and Results

Analysis of Time Spent in Open Arm: Time spent in open arm was noted for a total duration of 300 seconds after drug treatments. Intraperitoneal (i.p) administration of standard drug Diazepam in the dose of 2 mg/kg treatment caused statistically significant increase in the time spent in open arm (122.50 ± 9.04 sec) in comparison to control (NS) group (47.66 ± 2.19 sec). However, there is no much increase in time spent in open arm in the Diazepam 1 mg/kg treatment group (56.00 ± 3.88 sec) in comparison to control group (47.66 ± 2.19 sec).

Zinc oxide treatment group at the dose of 15 mg/kg (i.p) caused statistically significant increase in the time spent in open arm (113.33 ± 3.6 sec) in comparison to control (NS) group (47.66 ± 2.19 sec). However, there is no much increase in time spent in open arm in the zinc oxide 7.5 mg/kg and 30 mg/kg treatment group (51.00 ± 2.21 sec and 50.50 ± 4.92 sec respectively) compared to control group (47.66 ± 2.19 sec)

But, combination treatment of low doses of zinc oxide 7.5 mg/kg and Diazepam 1 mg/kg (i.p) showed significant increase in the time spent in open arm (78.67 ± 2.98 sec) as compared to the control (NS) group (47.66 ± 2.19 sec). Time spent in open arm in zinc oxide 15 mg/kg treatment group was comparable to Diazepam 2 mg/kg treatment (ZnO15 vs D2, p=0.16)

Time spent in open arm in combination group (ZnO7.5 + D1) (78.67 ± 2.98 sec) is significantly more than the zinc oxide 7.5 mg/kg alone (51.0 ± 2.21 sec) or Diazepam 1 mg/kg (56.0 ± 3.88 sec) alone.

Analysis of Time Spent in Closed Arm: Analysis of time spent in closed arm showed that there is statistically significant decrease in the time spent in closed arm in zinc oxide 15 mg/kg (185.33 ± 4.34 sec), Diazepam 2 mg/kg (177.5 ± 9.04 sec) treated groups than the control group (252.33 ± 2.11 sec) Combination of low doses of Zinc Oxide 7.5 mg/kg + Diazepam 1 mg/kg also caused significant

decrease in time spent in closed arm (221.33 ± 2.98 sec) than the zinc oxide 7.5 mg/kg alone (243 ± 2.2 sec) or diazepam 1 mg/kg alone (244 ± 3.88 sec)

Analysis of Number of Entries Into Open Arm: There is statistically significant increase in the number of entries into open arm in zinc oxide 15 mg/kg (3.5 ± 0.23) and Diazepam 2 mg/kg (3.83 ± 0.36) treated groups in comparison to control group (1.17 ± 0.38). Combination of low doses of Zinc Oxide (7.5 mg/kg, i.p) and Diazepam (1 mg/kg, i.p) showed significant increase in number of entries into open arm (3.0 ± 0.36) than the zinc oxide 7.5mg/kg (1.0 ± 0.26) alone or diazepam 1 mg/kg (1.33 ± 0.21) alone.

Number of entries into open arm in zinc oxide 15 mg/kg treatment is comparable to Diazepam 2 mg/kg treatment (ZnO15 vs D2, p=0.448)

Analysis of Number of Entries into Closed Arm: There is statistically significant increase in the number of entries into closed arm also in zinc oxide 15 mg/kg (5.0 ± 0.51) and Diazepam 2 mg/kg (5.33 ± 0.61) treatment groups in comparison to control group (4.33 ± 0.33). Number of entries in closed arm in zinc oxide 7.5 mg/kg + diazepam 1 mg/kg (4.16 ± 0.31) is significantly more than the zinc oxide 7.5 mg/kg alone (3.00 ± 0.26) or control group (3.33 ± 0.33) but no significant difference in comparison to diazepam 1 mg/kg group (4.33 ± 0.33)

Number of entries into closed arm in zinc oxide 15 mg/kg treatment is comparable to Diazepam 2 mg/kg treatment (ZnO15 vs D2, p=0.56).

Table 2: Comparison of number of entries of different groups in Elevated Plus Maze model (N=42)

Group (n=6) in each group	Drug	Dose in mg/ kg(i.p.)	Number of entries into open arm in 300 sec	Number of entries into closed arm in 300 sec
I control	NS	0.5 ml	1.17±0.38	3.33±0.33
II std	Diazepam	1	1.33 ± 0.21	4.33±0.33
III std	Diazepam	2	3.83 ± 0.36**	5.33±0.61**
IV test	Zinc Oxide	7.5	1.00 ± 0.26	3.0 ± 0.26
V test	Zinc Oxide	15	3.5 ± 0.23**	5.0 ± 0.51*
VI test	Zinc Oxide	30	1.8 ± 0.30	4.5 ± 0.341
VII combination	Zinc Oxide + Diazepam	7.5 + 1	3.0 ± 0.36**	4.16 ± 0.31

Table 3: Comparison of time spent of different groups in Elevated Plus Maze model (N=42)

Group (n=6) in each group	Drug	Dose in mg/kg(i.p.)	Time spent in open arm in 300 sec	Time spent in closed arm in 300 sec
I control	NS	0.5 ml	47.66 ± 2.19	252.33±2.11
II std	Diazepam	1	56.00 ± 3.88	244.0 ± 3.88
III std	Diazepam	2	122.50±9.04**	177.5±9.04**
IV test	Zinc Oxide	7.5	51.00 ± 2.21	249.0 ± 2.2
V test	Zinc Oxide	15	113.33 ± 3.6**	185.33±4.34**
VI test	Zinc Oxide	30	50.5 ± 2.01	249.5 ± 2.01

Note: All values are in Mean ± SEM * p< 0.05 & ** p <0.001 in comparison to control group. Test applied is ANOVA followed by Post hoc LSD

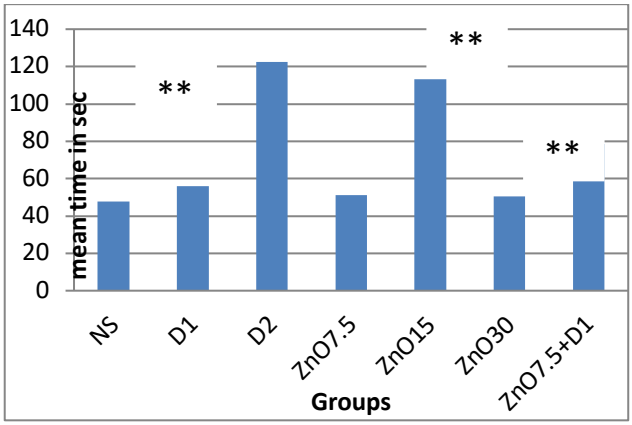

Figure 3: Bar diagram showing comparison of mean time spent in open and arm between the groups in elevated plus maze model

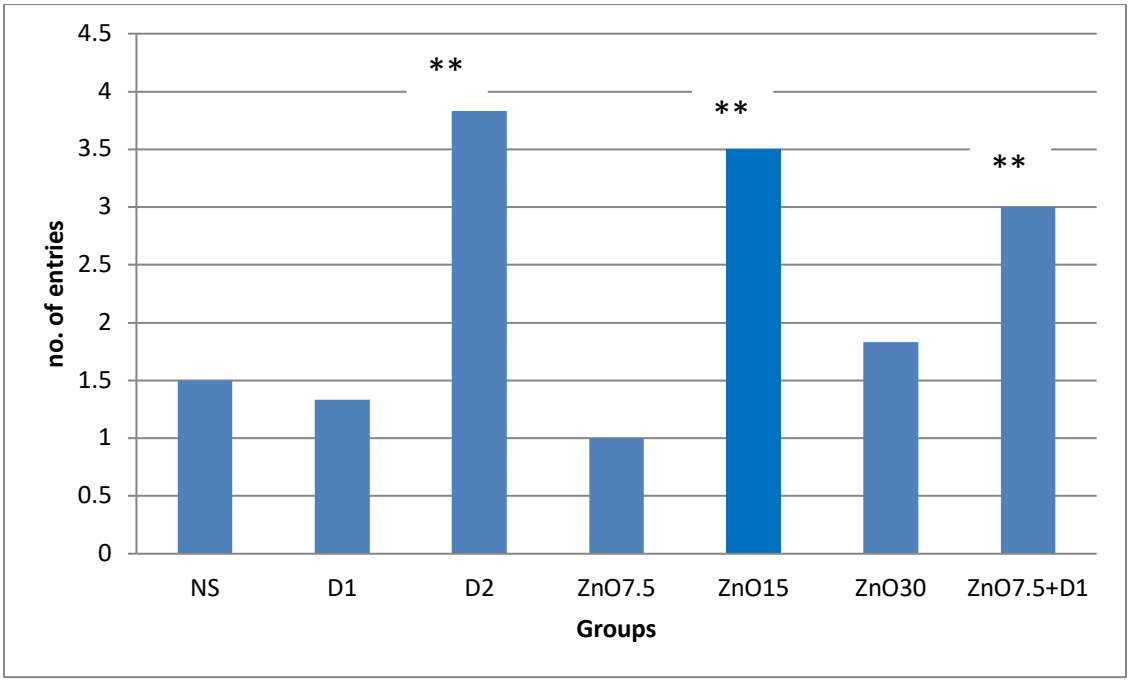

Figure 4: Bar diagram showing comparison of mean no. Of entries in open arm between the groups in elevated plus maze model

Note: All values are in Mean. * $p< 0.05$ & ** $p< 0.001$ in comparison to control group. Test applied is ANOVA followed by Post hoc LSD

NS – Normal saline, D1 – Diazepam 1 mg/kg, D2 – Diazepam 2 mg/kg, ZnO 7.5–Zinc Oxide 7.5 mg/kg, ZnO15 - Zinc Oxide 15mg/kg, ZnO30- Zinc Oxide 30mg/kg, ZnO7.5 + D1 –Zinc Oxide 7.5 mg/kg + Diazepam 1 mg/kg

Discussion

Recent research in zinc had led to the understanding of its role in anxiety. Hence the present study was undertaken to evaluate the anti-anxiety activity of zinc in albino rats using elevated plus maze model. Increase in the time spent in open arm, increased entries into the open arm and decreased time spent in closed arm are considered as parameters for assessing anti-anxiety effect of a drug in elevated plus maze model.

In the present study, it was found that zinc oxide in the dose of 15 mg/kg produced significant increase in time spent in open arm (113.33 ± 3.6 sec) in comparison to control (NS) group (47.66 ± 2.19 sec). Further, this dose of zinc oxide also significantly increased the number of entries into open arm (3.55 ± 0.23) in comparison to control (NS) (1.17 ± 0.38). In addition, zinc oxide in the doses of 15mg/kg significantly decreased the time spent in closed arm (185.33 ± 4.34 sec) in comparison to control (252.33 ± 2.11 sec). All these observations (Table no. 10,11) suggest zinc oxide in the dose of 15 mg/kg have anti-anxiety effect in elevated plus maze model.

The findings of the present study are similar to Samardzie et al. (2013) who studied the effect of zinc in elevated plus maze model in Wistar rats at the doses of 10, 20 and 30 mg/kg. The groups of rats receiving 20 mg/kg zinc spent significantly more time in the open arms of the maze, indicating an acute anxiolytic effect. The results are tabulated below.

The possible mechanism of action of zinc as anxiolytic is, when zinc is co-released with glutamate in the presynaptic spaces, it acts as an inhibitory neuromodulator of glutamate signaling. A number of studies have indicated that glutamate is an important element in anxiety and anxious behaviour and hence, blocking of glutamate N-Methyl D-Aspartate (NMDA) receptors can elicit a significant anxiolytic effect.

Electrophysiological studies have shown that zinc weakens the NMDA receptor-mediated response by two different mechanisms: [7]

- Non-competitive (allosteric) inhibition, responsible for reducing the channel-opening frequency.
- Open blocking effect.
- The other possible mechanism of anxiolytic effect by zinc is by increasing the release of Gamma Amino Butyric Acid (GABA) from the inter neurons of hippocampus, thus increasing the inhibitory effect of neurotransmitter

and leading to decrease in pre synaptic release of glutamate. [10]

The standard anti-anxiety drug diazepam in the dose of 2 mg/kg, intraperitoneally (i.p) significantly increased the time spent in open arm (122.5 ± 9.04 sec), increased number of entries into open arm (3.83 ± 0.36) and decreased the time spent in closed arm (177.5 ± 9.04 sec) in comparison to control group (47.66 ± 2.19 sec, 1.17 ± 0.38, 252.33 ± 2.11 sec respectively) indicating anti-anxiety effect. Diazepam 1 mg/kg did not produce the antianxiety effect.

Diazepam 2 mg/kg, i.p also significantly increased the time spent in light arena (212.0 ± 7.7 sec), increased number of entries into light arena (4.0 ± 0.36) and decreased time spent in dark arena (388 ± 7.7 sec) in comparison to control group (120.33 ± 3.89 sec, 1.5 ± 0.22, 479.67 ± 3.89 sec respectively) indicating anti-anxiety activity.

Time spent in the open arm in diazepam 2 mg/kg and zinc oxide 15 mg/kg groups (113.33 ± 3.6, 122.5 ± 9.04 sec respectively) is comparable {p=0.16}. Further, the difference in time spent in the closed arm in diazepam 2 mg/kg and zinc oxide 15 mg/kg groups (177.5 ± 9.04, 185.33 ± 4.34 sec respectively) is not significant. In addition, numbers of entries into open arm in diazepam 2 mg/kg, zinc oxide 15 mg/kg (3.83 ± 0.36, 3.5 ± 0.23 respectively) are not significantly different. So all these results indicate zinc oxide is equally efficacious to diazepam as anti-anxiety drugs in elevated plus model.

The potentiating effect of zinc oxide and diazepam can be explained as follows. Both these drugs act by different mechanisms. Diazepam is known to act on $GABA_A$ type of receptors (benzodiazepine binding site) and increase chloride ion permeability leading to neuronal depression which might be responsible for anti-anxiety action whereas zinc causes post synaptic inhibition of NMDA receptors either by allosteric inhibition or direct blockade.[10]

Conclusion

The present study was conducted to evaluate antianxiety activity of zinc oxide in various doses using elevated plus maze. It was found that zinc oxide at dose of 15mg/kg and diazepam 2mg/kg had produced antianxiety action. This was evident by increase in number of entries in open arm, time spent in open arm and decrease in time spent in closed arm. Zinc oxide acts as anxiolytic by possible inhibition of post synaptic NMDA receptors. Combination of low dose zinc oxide 7.5 mg/kg and diazepam 1 mg/kg also produced antianxiety activity, which was not observed with diazepam 1 and zinc oxide 7.5 mg/kg alone. This shows that zinc oxide in low dose can potentiate the effect of antianxiety action of diazepam 1 mg/kg. The reason for this potentiation may be different mechanism of action of diazepam and zinc oxide. However, further studies are needed to confirm the antianxiety effect of zinc oxide using other models of anxiety and to explore the role of zinc as therapeutic tool in other central nervous system disorders.

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