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Potency-Specific Anxiolytic Effects Of Homeopathic Plumbum Metallicum In A Murine Light-Dark Box Model

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Abstract:

Background:

The light-dark box test is a widely used behavioural model for assessing anxiety in rodents, based on their aversion to bright areas and preference for dark, enclosed spaces. Plumbum metallicum, a classical homeopathic remedy with known neurobehavioral indications, was evaluated for anxiolytic-like effects using this model.

Objective:

To investigate the anxiolytic potential of Plumbum metallicum in mice at different potencies (6C, 30C, 200C) and compare its effects with standard anxiogenic (PTZ) and anxiolytic (diazepam) controls.

Methods:

Adult male Swiss Albino mice(n=36) were divided into six groups: Control, PTZ (80 mg/kg), Diazepam (4 mg/kg) + PTZ, and Plumbum metallicum (6C, 30C, 200C) + PTZ. Remedies were administered orally for 14 days prior to PTZ administration. Behavioral parameters recorded included time in light, number of transitions, latency to enter the dark compartment, and time spent in darkness.

Results:

PTZ significantly increased anxiety-like behavior, while diazepam reversed these effects. Plumbum metallicum showed a clear potency-dependent anxiolytic effect. Mice treated with 6C, 30C, and 200C spent 65, 95, and 135 seconds in the light respectively, made 4, 7, and 10 transitions, showed latencies of 6, 9, and 13 seconds to enter the dark compartment, and spent 195, 165, and 125 seconds in the dark.

Conclusion:

Chronic administration of Plumbum metallicum, especially at 200C, significantly reduced anxiety-like behavior in mice, with results comparable to diazepam. These findings support the potential of high-potency homeopathic remedies in neurobehavioral research using validated models.

Key words: *Plumbum metallicum ,Homeopathy,Anxiety,Light-dark box test*

INTRODUCTION

The light-dark box test relies on rats' inherent aversion to illuminated areas and their instinctive exploratory behavior when confronted with mild stressors, including light and unfamiliar environments.[1] The test has demonstrated strong reliability and sensitivity to a range of anxiolytic compounds, including benzodiazepines [2]

Homeopathy is a system of medical therapeutics which has about 351 remedies which can treat Anxiety.[3] Significant behavioural effects have been observed in rodent studies using homeopathic preparations like Gelsemium sempervirens, Pulsatilla nigricans, Ignatia amara and Anat Imperator particularly within established models such as the open field and light/dark box tests [4–7].These findings have spurred growing interest in evaluating the neurobehavioral effects of homeopathic medicines using conventional animal models.

In the present study, Plumbum metallicum, a classical homeopathic remedy known for its neurological and behavioral indications, is investigated for its anxiolytic-like effects using the light-dark box test in rodents.The present study investigates the anxiety-reducing potential of the homeopathic remedy Plumbum metallicum in mice by examining its effects at multiple potencies using the Light-Dark Box test, benchmarking its efficacy against standard agents (PTZ and diazepam), and contributing to the scientific appraisal of homeopathic interventions through validated behavioral models.

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REVIEW OF LITERATURE-

In homeopathy, locomotor and anxiolytic effects are not explicitly referenced within the repertory. Instead of prescribing based on a specific diagnosis, homeopathic treatment focuses on the individualized symptoms presented by the patient. In homeopathy, remedies are selected based on their ability to match the patient's overall symptom profile.For cases involving symtoms such as locomotor and anxiolytic effects related to seizures the remedy most commonly indicated in the repertory is Plumbm Metallicum. However, this does not imply that the this remedy is universally applicable to all such cases .It represents the nearest similimum — a remedy that most closely matches the patient's individual symptom presentation.

Also given below are the Repertory Sheets of the remedy and the rubrics covering the remedy for rubrics like Seizures, Convulsion and Epilepsy and Mind Chapter.

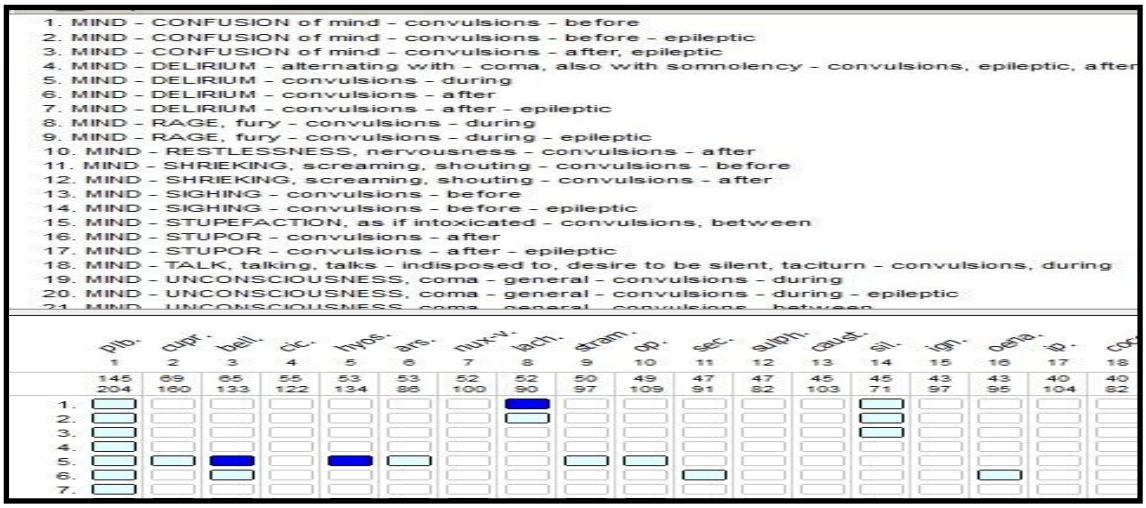


Figure 1 Synthesis Repertory Mind ChapterChapter

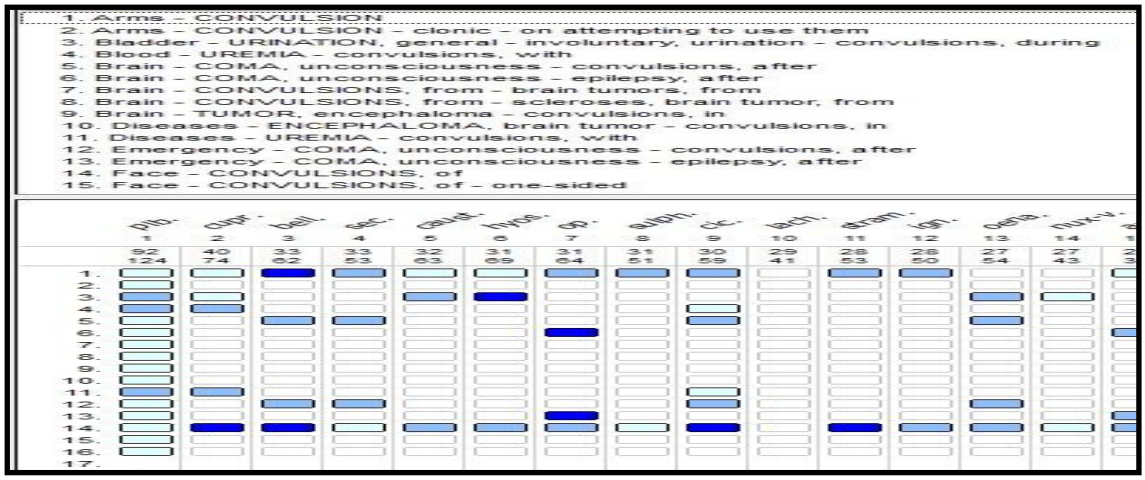


Figure 2 Murphy Repertory Neurological Symptoms Chapter

MATERIALS AND METHODS-

Animals :

Adult albino Wistar male mice (100-150 g) were used for this study.

The animals were housed at 24 ±2°C and relative humidity 55 ±5 with 12:12 h light and dark cycle. They had a free access to food and water ad libitum.

The animals were acclimatized for a period of 7 days before the study.

Ethical Approval

The experimental protocol was approved by the Institutional Animal Ethics Committee of SMBT College of Pharmacy, Nashik. (Registration no: 1329/PO/Re/S/10/CCSEA).

Drugs and Chemicals

1. PTZ (Pentylenetetrazol): 80 mg/kg i.p. to induce seizures
2. Plumbum metallicum: Homeopathic preparations (6C, 30C, and 200C potencies)
3. Diazepam: Standard anticonvulsant (4 mg/kg i.p.)

Homeopathic Drug Preparation and Administration Protocol-

For experimentation the experimental animals were given the homeopathic drug Plumbum metallicum at three different dose (potencies) for 14 days. On 14th Day PTZ was given to the experimental animals and readings were noted. After readings the animals were sacrificed and brain was extracted for essays.

Initially, an acute administration protocol was attempted in which the homeopathic dilutions were administered shortly before PTZ injection on the same day. However, this acute intervention failed to elicit anticonvulsant activity, as animals displayed no significant delay in seizure onset.

In response, and consistent with the laws of Drug proving and the Theory of chronic disease management, a chronic dosing protocol was adopted. This allowed for potential systemic modulation and subtle energetic imprinting over time.

Experimental Design

Number of mice in each group- 6

Gender- All Male.

4. Group I: Control (normal saline)
5. Group II: PTZ (80 mg/kg i.p.)
6. Group III: Diazepam + PTZ
7. Group IV: Plu M(Plumbum Metallicum) 6C + PTZ
8. Group V: Plu M 30C + PTZ
9. Group VI: Plu M 200C + PTZ

Total number of mice- 36

Behavioural Test-

The "light-dark box test" is a behavioral model used in neuroscience to assess anxiety-like behavior in rodents by observing their movement between a lit (aversive) and darkened (safe) compartment.

Purpose : the light-dark box test is a simple apparatus consisting of a chamber divided into two compartments: one brightly lit and open, and the other dark and enclosed.

Procedure : Rodents are placed in the middle of the brightly illuminated chamber and allowed to move freely between the two compartments.

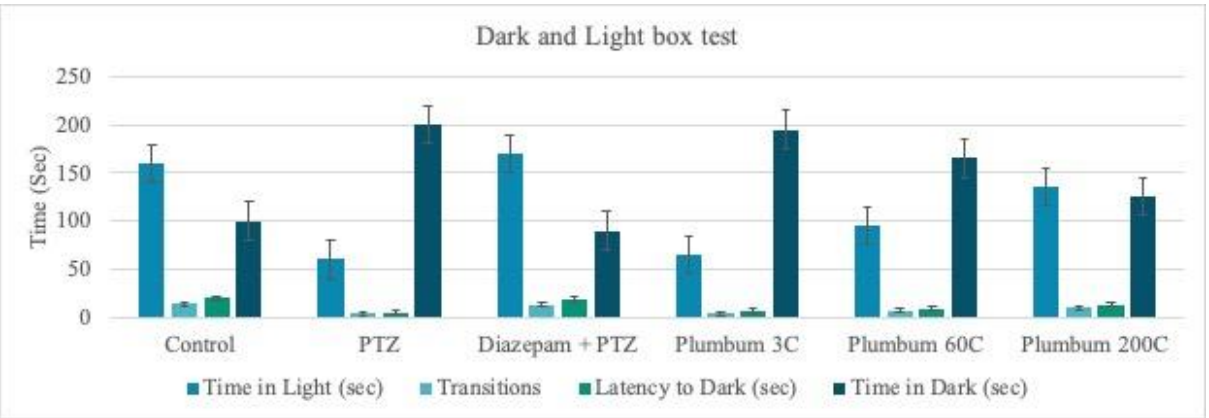
RESULTS

Group	Time in Light (sec)	Transitions	Latency to Dark (sec)	Time in Dark (sec)
Control	160	14	20	100
PTZ	60	4	5	200
Diazepam + PTZ	170	13	18	90
Plumbum 3C	65	4	6	195
Plumbum 60C	95	7	9	165

Plumbum 200C	135	10	13	125

Group	**Anxiety Level**	**Interpretation**
PTZ	High	Strong anxiogenic effect
Diazepam + PTZ	Very Low	Standard anxiolytic effect
Cuprum 3C	Mild	Weak effect
Cuprum 60C	Moderate	Effective
Cuprum 200C	Strong	Comparable to Diazepam
Plumbum 3C	Mild	Limited efficacy
Plumbum 60C	Moderate	Better than 3C
Plumbum 200C	Strong	Significant anxiolytic effect

Graph 1 : Effect of Plumbum Metallicum on Dark and Light box test



All the data were shown as mean ± standard error of the mean. Statistical analysis was performed with one-way ANOVA followed by Dunnett's test.

DISCUSSION

This study evaluated the potential anxiolytic-like effects of the homeopathic remedy Plumbum metallicum across three different potencies (6C, 30C, 200C) using the light-dark box test, a validated behavioral model for anxiety assessment in rodents. The light-dark box paradigm is grounded in rodents' natural preference for dark environments and their tendency to explore new surroundings, making it a reliable indicator of anxiety-related behavior.

In the present experiment, PTZ-treated mice showed significantly heightened anxiety-like behaviors, as evidenced by reduced time spent in the light compartment and fewer transitions, confirming its anxiogenic action. Diazepam, a standard anxiolytic, effectively reversed these behaviors, thereby validating the model's responsiveness. While the 6C potency showed negligible behavioral improvements, the 30C potency produced moderate anxiolytic-like effects. The most pronounced changes were observed in the

group receiving the 200C potency, which showed a substantial increase in exploratory behavior and time spent in the light area, suggesting a reduction in anxiety levels comparable to the diazepam group.
It is important to note that acute administration of the remedy did not yield significant results, highlighting the relevance of chronic dosing in homeopathy. This aligns with the core principles of homeopathic therapeutics, which often emphasize repeated dosing over time to evoke a system-wide response in Chronic diseases.

CONCLUSION

The present research demonstrates that chronic administration of Plumbum metallicum, particularly at 200C potency, significantly reduces anxiety-like behaviors in mice, as assessed by the light-dark box test. The behavioral changes observed were comparable to those elicited by the standard anxiolytic diazepam, supporting the remedy's potential as an anxiolytic agent.
These findings contribute to the growing body of evidence supporting the use of homeopathic medicines in behavioral pharmacology and highlight the value of standardized animal models for evaluating their efficacy. Continued research using larger cohorts and mechanistic studies will be essential to substantiate these results and deepen our understanding of the therapeutic potential of homeopathy in neurobehavioral conditions.

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