

PRELIMINARY EVALUATION OF ANXIOLYTIC-LIKE ACTIVITY OF SELECTED
ANTI-HISTAMINIC DRUGS USING THE ELEVATED PLUS MAZE MODEL IN WISTAR
ALBINO RATSShanmugapriya M.*¹, Sivakumar G. M.², Perumal P.³, Abishek R. K.⁴, Arunraj K.⁴, Gobika R.⁴, Shaveetha A.⁴,
Shibi S. R.⁴¹Assistant Professor, Department of Pharmacology, JKK Munirajah Institute of Health Sciences College of Pharmacy,
T. N. Palayam – 638 506.²Associate Professor, Department of Pharmacology, JKK Munirajah Institute of Health Sciences College of Pharmacy,
T. N. Palayam – 638 506.³Professor cum Principal, Department of Pharmaceutical Chemistry, JKK Munirajah Institute of Health Sciences
College of Pharmacy, T. N. Palayam – 638 506.⁴Department of Pharmacology, JKK Munirajah Institute of Health Sciences College of Pharmacy, T. N. Palayam – 638
506.***Corresponding Author: Shanmugapriya M.**Assistant Professor, Department of Pharmacology, JKK Munirajah Institute of Health Sciences College of
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ABSTRACT

Background: Anxiety disorders remain a significant neuropsychiatric burden, and limitations of existing anxiolytic drugs have encouraged interest in repurposing established medications. Antihistamines differ in central nervous system penetration, suggesting possible variation in anxiety-related behavioral effects. **Objective:** To evaluate and compare the anxiolytic-like activity of cetirizine and promethazine using the Elevated Plus Maze model in Wistar albino rats, with diazepam as the standard reference drug. **Methods:** Adult Wistar albino rats were divided into four groups with six animals in each group. The control group received normal saline; the standard group received diazepam 3 mg/kg; and the test groups received cetirizine 2 mg/kg and promethazine 2 mg/kg. Treatments were administered orally, and behavioral assessment was performed 60 minutes after administration. Time spent in open-arm entries, closed-arm entries, and total-arm entries was recorded. Data were expressed as mean $\pm$ SD and analyzed using one-way ANOVA followed by Tukey's post hoc test. **Results:** Control animals showed the lowest open arm time and open arm entries. Diazepam showed the highest open-arm exploration and promethazine showed higher responses than cetirizine. Open arm time increased from 87.75 ± 5.57 sec in control animals to 149.25 ± 14.70 sec with diazepam, 139.62 ± 9.62 sec with promethazine, and 118.40 ± 2.98 sec with cetirizine. Total arm entries were comparable to or higher than control across treatment groups. **Conclusion:** Promethazine and cetirizine demonstrated anxiolytic-like effects, with promethazine showing stronger activity. These hypothesis-generating findings warrant further investigation of antihistamines in anxiety-related behavioral models.

KEYWORDS: Anxiety; Histamine H1 Antagonists; Drug Repositioning; Diazepam; Cetirizine; Promethazine.**INTRODUCTION**

Anxiety disorders remain a major neuropsychiatric burden, significantly affecting quality of life and functional outcomes.^[1,2] Although pharmacological treatments such as benzodiazepines and selective serotonin reuptake inhibitors are available, their use is

often limited by incomplete response, delayed onset, sedation, dependence, and tolerability concerns.^[3,4] The Elevated Plus Maze (EPM) is a widely used rodent model for assessing anxiety-like behavior, where increased open-arm time and open-arm entries indicate reduced anxiety-like response, while total arm entries

help assess locomotor influence.^[5,6] Histamine is an important central neuromodulator involved in arousal, cognition, wakefulness, and neuropsychiatric regulation.^[7,8] Antihistamines differ in central nervous system penetration; first-generation agents show greater blood-brain barrier penetration and sedative potential, whereas second-generation agents such as cetirizine generally show lower CNS activity.^[9,10] However, comparative experimental evaluation of first- and second-generation antihistamines in standardized anxiety models remains limited. Drug repurposing provides a rational strategy for identifying new therapeutic applications of existing drugs with known pharmacological profiles.^[11,12] Recent repurposing approaches have also been explored specifically for anxiety-related therapeutics.^[13] Therefore, the primary aim of the present study was to evaluate and compare the anxiolytic-like activity of cetirizine and promethazine using the EPM model in Wistar albino rats, with diazepam as the standard reference drug.

MATERIALS AND METHODS

Experimental Animals

Adult Wistar albino rats of either sex, weighing 150–200 g, were used in the study. The exact sex distribution within each treatment group was not recorded separately; therefore, sex was not included as an analytical covariate and this has been acknowledged as a limitation. The animals were maintained under standard laboratory conditions ($22 \pm 2^\circ\text{C}$, 12 h light/dark cycle) with free access to food and water. Animals were acclimatized for at least one week prior to experimentation. The use of rodents under controlled environmental conditions for behavioural assessment in anxiety models is well established in experimental pharmacology.^[5]

Experimental Design and Treatment

Animals were randomly allocated into four groups using a random-number method, with six animals in each group. A sample size of six animals per group was selected because the present work was designed as a preliminary exploratory behavioural pharmacology study using the Elevated Plus Maze, a widely used behavioural screening model for anxiety-like responses in rodents.^[5,14] This group size was chosen to minimize animal use while allowing exploratory comparison among treatment groups. However, because no formal *a priori* power calculation was performed, the findings should be interpreted as preliminary and hypothesis-generating, consistent with transparent reporting expectations for animal research.^[15]

The control group received normal saline (0.9% NaCl), while the standard group received diazepam (3 mg/kg). The test groups were treated with cetirizine (2 mg/kg) and promethazine (2 mg/kg), respectively. The 2 mg/kg doses of cetirizine and promethazine were selected as exploratory screening doses to compare second- and first-generation antihistaminic agents under identical experimental conditions. Because published EPM-

specific dose-response data for these agents are limited, the selected dose should be regarded as empirical rather than pharmacodynamically equivalent. Therefore, the present design cannot determine the optimal anxiolytic-like dose range or allow direct potency comparison between cetirizine and promethazine. Drug doses were calculated according to the individual body weight of each animal on the day of administration. Cetirizine and promethazine were freshly prepared in normal saline, and the control group received an equivalent oral volume of vehicles. All treatments were administered via the oral route, and behavioural assessment was carried out 60 minutes after drug administration.

Method for Elevated Plus Maze (EPM)

The anxiolytic-like activity was evaluated in the Elevated Plus Maze (EPM), a validated behavioural paradigm for the evaluation of anxiety-related responses in rodents. The EPM apparatus consisted of two open arms and two closed arms in a plus-shaped configuration and elevated from the floor. Exact dimensions of the apparatus were not provided in the study report and were identified as a limitation for reproducibility. Animals were habituated to the experimental room prior to testing but were not pre-exposed to the Elevated Plus Maze apparatus prior to the formal 5 min test session. During testing, each animal was placed at the center of the maze facing an open arm and allowed to explore freely for 5 minutes. The time spent in open arms, number of open arm entries, and number of closed arm entries were recorded. An entry was defined as the placement of all four paws in an arm. Behavioural observations were recorded by an investigator blinded to treatment allocation. The apparatus was cleaned between trials to minimize olfactory cues. Because EPM performance can be influenced by test experience, illumination, and other environmental conditions, efforts were made to maintain similar testing conditions across animals.^[16,17] Although testing was conducted under comparable lighting conditions across animals, the exact illumination intensity in lux was not recorded. Increased open arm exploration is considered indicative of reduced anxiety-like behaviour.^[14] Further methodological standardization of EPM testing procedures has been described in widely cited protocols.^[5]

Primary and Secondary Outcomes

Time spent in open arms was considered the primary endpoint for assessing anxiolytic-like activity. Open arm entries and closed arm entries were considered secondary behavioural outcomes. Total arm entries were used as a supporting locomotor activity measure.

Assessment of Locomotor Activity

Total arm entries were calculated as the sum of open and closed arm entries and used as an index of general locomotor activity. This parameter was used to help differentiate anxiolytic-like effects from sedative or motor-impairing effects.^[14]

Statistical Analysis

Data were expressed as mean $\pm$ SD ($n = 6$). One-way analysis of variance (ANOVA) was performed separately for each outcome variable. Tukey's post hoc test was used for pairwise comparisons among the control, diazepam, cetirizine, and promethazine groups. Effect size was estimated using eta-squared (η^2) for one-way ANOVA outcomes. Differences were considered statistically significant at $p < 0.05$. The use of ANOVA for comparing multiple treatment groups is standard in behavioural pharmacology studies.^[5]

Ethical Considerations

All procedures were conducted in accordance with institutional guidelines for animal care and use and were

approved by the Institutional Animal Ethics Committee (Approval No.: 688/2/C-CPCSEA/SM/20260314).

RESULTS

Effect of Antihistaminic Drugs on Time Spent in Open Arms

Time spent in the open arms was analyzed as the primary endpoint for assessing treatment effects in the Elevated Plus Maze (Table 1, Figure 1). The control group showed the lowest open arm time (87.75 ± 5.57 sec), while diazepam showed the highest open arm time (149.25 ± 14.70 sec). When expressed as percentage of the 300-second test session, open arm time was 29.25% in the control group, 49.75% in the diazepam group, 39.47% in the cetirizine group, and 46.54% in the promethazine group.

Table 1: Effect of antihistaminic drugs on time spent in open arms in the Elevated Plus Maze model.

Sl. No.	Group	Treatment	Open Arm Time (sec) (Mean $\pm$ SD)	% Change vs Control
1	Control	Vehicle	87.75 ± 5.57	—
2	Standard	Diazepam	149.25 ± 14.70	+70.1%
3	Test 1	Cetirizine	118.40 ± 2.98	+34.9%
4	Test 2	Promethazine	139.62 ± 9.62	+59.1%

Values are expressed as mean $\pm$ SD ($n = 6$). Percentage change versus control was calculated as: $[(\text{Treatment mean} - \text{Control mean}) / \text{Control mean}] \times 100$. EPM, Elevated Plus Maze. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Differences were considered significant at $p < 0.05$.

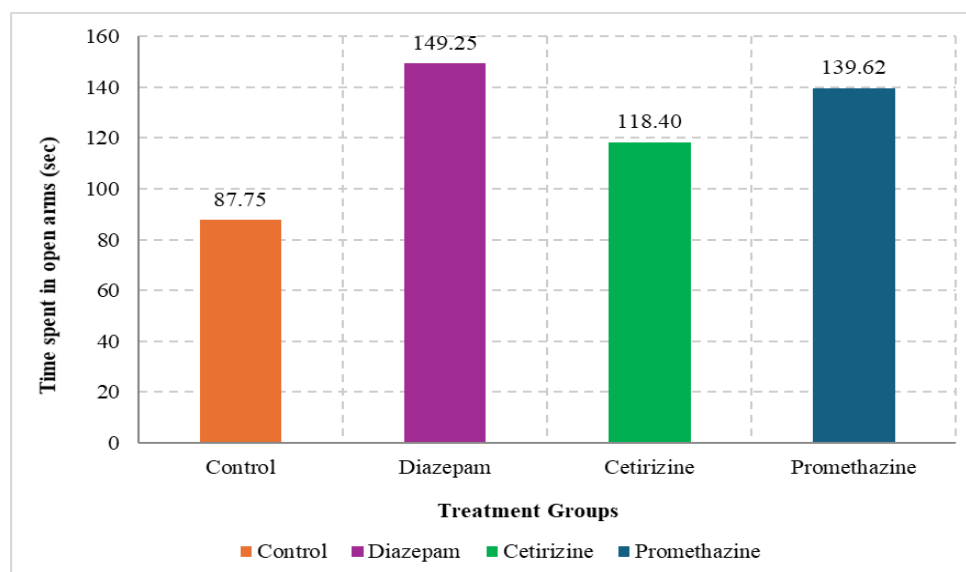


Figure 1: Effect of antihistaminic drugs on time spent in open arms in the Elevated Plus Maze model.

Among the antihistaminic drugs, promethazine showed higher open arm time (139.62 ± 9.62 sec) than cetirizine (118.40 ± 2.98 sec). The observed order of open arm time was Control < Cetirizine < Promethazine < Diazepam. Compared with the control group, all treatment groups showed statistically significant increases in open arm time ($p < 0.05$).

Effect on Open Arm Entries

Open arm entries were analyzed as a secondary behavioural outcome (Table 2). The control group

showed the lowest number of open arm entries (2.50 ± 0.55), while diazepam showed the highest number of entries (5.67 ± 0.52).

Table 2: Effect of antihistaminic drugs on open arm entries and total arm entries in the Elevated Plus Maze model.

Sl. No.	Group	Open Arm Entries (Mean $\pm$ SD)	Closed Arm Entries (Mean $\pm$ SD)	Total Entries (Mean $\pm$ SD)
1	Control	2.50 $\pm$ 0.55	6.00 $\pm$ 0.63	8.50 $\pm$ 0.55
2	Diazepam	5.67 $\pm$ 0.52	5.17 $\pm$ 0.41	10.67 $\pm$ 0.52
3	Cetirizine	3.67 $\pm$ 0.52	5.50 $\pm$ 0.55	9.17 $\pm$ 0.75
4	Promethazine	4.67 $\pm$ 0.52	5.83 $\pm$ 0.41	10.50 $\pm$ 0.55

Values are expressed as mean $\pm$ SD ($n = 6$). Total entries were calculated as open arm entries + closed arm entries and used as an index of locomotor activity. EPM, Elevated Plus Maze.

Promethazine-treated animals showed higher open arm entries (4.67 $\pm$ 0.52) than cetirizine-treated animals (3.67 $\pm$ 0.52). The trend in open arm entries was consistent with the open arm time results, with the order Control < Cetirizine < Promethazine < Diazepam.

As an additional descriptive EPM index, the percentage of open arm entries was calculated as open arm entries divided by total entries $\times$ 100. The %OAE was 29.41% in control, 53.14% in diazepam, 40.02% in cetirizine, and 44.48% in promethazine groups.

Total Arm Entries and Assessment of Locomotor Activity

Total arm entries were analyzed as a supporting measure of locomotor activity (Table 2). The control group showed 8.50 $\pm$ 0.55 total entries, while diazepam,

cetirizine, and promethazine groups showed 10.67 $\pm$ 0.52, 9.17 $\pm$ 0.75, and 10.50 $\pm$ 0.55 total entries, respectively.

No reduction in total arm entries was observed in the treatment groups compared with control. This pattern indicates that treatment-related increases in open arm measures occurred without an observed decrease in total arm activity. For total arm entries, one-way ANOVA showed a significant group effect [$F(3,20) = 18.37$, $p < 0.001$]; however, no treatment group showed a reduction in total entries compared with control. Tukey's post hoc analysis showed that total arm entries were significantly higher in the diazepam ($p < 0.001$) and promethazine ($p < 0.001$) groups compared with control, whereas cetirizine did not differ significantly from control ($p = 0.245$; Table 3).

Table 3: Tukey's post hoc pairwise comparisons for EPM behavioural outcomes.

Outcome	Pairwise comparison	Mean difference	Tukey adjusted p-value	Interpretation
Open arm time	Control vs Diazepam	61.50	<0.001	Significant
	Control vs Cetirizine	30.65	<0.001	Significant
	Control vs Promethazine	51.87	<0.001	Significant
	Diazepam vs Cetirizine	30.85	<0.001	Significant
	Diazepam vs Promethazine	9.63	0.308	Not significant
	Cetirizine vs Promethazine	21.22	0.004	Significant
Open arm entries	Control vs Diazepam	3.17	<0.001	Significant
	Control vs Cetirizine	1.17	0.005	Significant
	Control vs Promethazine	2.17	<0.001	Significant
	Diazepam vs Cetirizine	2.00	<0.001	Significant
	Diazepam vs Promethazine	1.00	0.018	Significant
	Cetirizine vs Promethazine	1.00	0.018	Significant
Total arm entries	Control vs Diazepam	2.17	<0.001	Significant
	Control vs Cetirizine	0.67	0.245	Not significant
	Control vs Promethazine	2.00	<0.001	Significant
	Diazepam vs Cetirizine	1.50	0.002	Significant
	Diazepam vs Promethazine	0.17	0.960	Not significant
	Cetirizine vs Promethazine	1.33	0.005	Significant

Values represent Tukey's post hoc pairwise comparisons following one-way ANOVA. Mean difference represents the absolute difference between group means; direction of effect should be interpreted from the group means presented in Tables 1 and 2. $p < 0.05$ was considered statistically significant. EPM, Elevated Plus Maze.

Comparative Pattern of Treatment Responses

Across the measured behavioural outcomes, diazepam showed the highest open arm exploration, followed by promethazine and cetirizine. The control group showed the lowest values for both open arm time and open arm entries. The overall observed pattern for open arm time

and open arm entries was Control < Cetirizine < Promethazine < Diazepam. One-way ANOVA showed significant group differences for open arm time [$F(3,20) = 51.11$, $p < 0.001$] and open arm entries [$F(3,20) = 39.74$, $p < 0.001$]. The effect size was large for open arm time ($\eta^2 = 0.88$), open arm entries ($\eta^2 = 0.86$), and total

arm entries ($\eta^2 = 0.73$). Tukey's post hoc analysis showed that diazepam, cetirizine, and promethazine differed significantly from control for both open arm time and open arm entries ($p < 0.05$). Detailed Tukey's post hoc pairwise comparisons for open arm time, open arm entries, and total arm entries are presented in Table 3. For the primary outcome, the mean open arm time was numerically lower in the promethazine group than in the diazepam group, but this difference did not reach statistical significance (mean difference: 9.63 sec; $p = 0.308$; Table 3). This non-significant result should not be interpreted as evidence of pharmacological equivalence.

DISCUSSION

Anxiolytic-Like Effects of Antihistamines: Evidence and Possible Mechanisms

The results of the present study showed that promethazine produced higher open-arm exploration than cetirizine, while diazepam showed the highest mean response. The non-significant difference between promethazine and diazepam in open arm time should be interpreted cautiously because the study was not designed or powered to test pharmacological equivalence. Cetirizine produced a lower but measurable increase in open-arm exploration compared with control. The differences in central nervous system activity between the two antihistaminic agents may account for their varying responses. Histamine is an important neuromodulator involved in arousal, cognition, wakefulness, and neuropsychiatric regulation.^[7]

The mean open arm time was numerically lower in the promethazine group than in the diazepam group, but this difference did not reach statistical significance ($p = 0.308$; Table 3). This finding should be interpreted with caution regarding pharmacological equivalence because the study was not designed or powered for equivalence testing. Diazepam also remained higher than promethazine for open arm entries ($p = 0.018$; Table 3), so this finding should not be interpreted as overall pharmacological equivalence. Recent findings also suggest a possible relationship between histaminergic signalling and anxiety- or fear-related behaviours mediated partly through H1 receptor-associated pathways.^[18] However, the present study did not include receptor-level or neurochemical analysis; therefore, the observed behavioural pattern should be interpreted as compatible with possible histaminergic modulation rather than as direct evidence of H1 receptor-mediated anxiolysis.

The stronger behavioural response observed with promethazine may be related to its first-generation antihistaminic profile and greater central nervous system activity. Promethazine has antihistaminic, antidopaminergic, anticholinergic, and sedative properties, which may contribute to broader central behavioural effects.^[10] In contrast, cetirizine, a second-generation antihistamine, is generally associated with

lower central nervous system penetration and fewer sedative effects.^[9]

Overall, the behavioural pattern observed in this experiment can be summarized descriptively as Control < Cetirizine < Promethazine < Diazepam. These findings should be considered hypothesis-generating and provide only preliminary behavioural evidence for further evaluation of antihistaminic drugs in anxiety-related models, while emphasizing that receptor-level and neurochemical studies are required before confirming the underlying mechanism.

Differentiation from Sedation: Locomotor Activity Considerations

A key issue in interpreting Elevated Plus Maze findings is whether increased open-arm exploration reflects anxiolytic-like activity or nonspecific changes in locomotor function. This is particularly relevant for antihistaminic drugs, as first-generation agents such as promethazine may produce central nervous system depressant and sedative effects.^[10] In the present study, total arm entries were not reduced in the diazepam, cetirizine, or promethazine groups compared with control, suggesting that the increased open-arm time and entries were not primarily driven by gross locomotor suppression. However, rodent anxiety-like behavioural assays require cautious interpretation because outcomes may be influenced by exploratory drive, locomotor function, sensory factors, and contextual testing variables.^[19]

Total arm entries were significantly higher in both the diazepam and promethazine groups compared with control, while cetirizine did not differ significantly from control (Table 3). This finding argues against gross locomotor suppression under the present experimental conditions. However, the increased total entries also suggest that altered exploratory drive, behavioural activation, or context-dependent motor activity may have contributed to the EPM profile. For promethazine, this is particularly important because its sedative, anticholinergic, and antidopaminergic properties could produce complex behavioural effects rather than simple locomotor suppression. Therefore, these findings should be interpreted cautiously and require confirmation using open-field and rotarod assessments. The control group spent 29.25% of the session in the open arms, which is relatively high for a naïve EPM control group and may reflect contextual, strain-related, environmental, or procedural influences. This should be considered when interpreting the magnitude of drug-induced changes.

This interpretation is supported by recent behavioural literature showing that anxiety-like behaviour, fear, and exploratory drive can represent partly dissociable components rather than a single behavioural construct.^[20] Recent EPM-focused literature also emphasizes that open-arm exploration should be interpreted alongside activity-related indices to avoid misclassifying altered

exploration or sedation as anxiolysis.^[6,21] Although preserved or increased total arm entries reduce concern for gross motor suppression, total entries alone cannot exclude subtle psychomotor, attentional, or exploratory-drive effects. Future studies should include open-field and rotarod testing for stronger confirmation.

Drug Repurposing and Translational Relevance

The present findings provide preliminary support for exploring selected antihistaminic drugs within a drug-repurposing framework. Drug repurposing is increasingly recognized as a practical strategy because existing drugs have known pharmacological and safety profiles, which may reduce cost, time, and uncertainty compared with *de novo* drug development.^[22] This approach is particularly relevant in neuropsychiatric research, where conventional drug discovery remains challenging and translation from preclinical models to clinical benefit requires careful validation.^[23,24] Recent anxiety-focused repurposing studies further support the value of identifying existing pharmacological agents that may warrant evaluation for anxiety-related indications.^[13]

In the present study, promethazine showed stronger anxiolytic-like activity than cetirizine, suggesting that first-generation antihistamines with greater central nervous system activity may produce more prominent behavioural effects. However, this potential must be balanced against known sedative, anticholinergic, and toxicity-related concerns associated with promethazine.^[10] Clinical evidence also indicates that sedating antihistamines such as promethazine may be used as anxiolytic or hypnotic agents in some settings, but safety concerns remain important when considering translational relevance.^[25] In contrast, cetirizine produced a weaker behavioural response but may be more favourable from a tolerability perspective because second-generation antihistamines generally show fewer central nervous system adverse effects compared with older first-generation agents.^[9] Therefore, these findings should be viewed as preliminary repurposing evidence rather than direct evidence for clinical anxiolytic use. Future translational studies should evaluate dose-response relationships, safety margins, chronic administration, and clinical relevance before therapeutic application.

Limitations and Future Directions

The present study provides preliminary behavioural evidence, but several limitations should be acknowledged. First, the study used a small sample size and assessed the drugs at a single dose and single acute time point, which limits confidence in estimating the magnitude, dose-response relationship, and persistence of the observed anxiolytic-like effects. No *a priori* power calculation was performed, and the small sample size may have reduced the ability to detect moderate pairwise differences. Therefore, non-significant pairwise comparisons, particularly between promethazine and

diazepam, should not be interpreted as evidence of equivalence. The large eta-squared values observed in this study should also be interpreted cautiously because effect-size estimates may be inflated in small exploratory samples. Second, the evaluation was restricted to the Elevated Plus Maze. Reliance on a single behavioural assay may restrict construct validity and generalizability because anxiety-like behaviour in rodents is best interpreted using convergent evidence from multiple paradigms.^[26,27] Third, total arm entries were used to assess locomotor influence, but this measure cannot fully exclude subtle psychomotor, attentional, or sedative effects. In addition, the exact sex distribution within each treatment group and illumination intensity during EPM testing were not recorded, which may limit reproducibility and restrict interpretation of sex-related or environment-related behavioural variation. Exact EPM apparatus dimensions were not available from the study record, which may further limit reproducibility because arm size, wall height, and elevation can influence exploratory behaviour.^[16,17,28]

Future studies should include dose-response experiments, repeated or chronic dosing models, and validation across multiple anxiety paradigms such as the light-dark box, open field test, and social interaction test. Comparative use of open field and EPM testing may help strengthen interpretation of anxiety-like behaviour and locomotor influence.^[27] Additional motor-control assessments, including open field and rotarod testing, would strengthen interpretation of locomotor independence. Mechanistic studies assessing histaminergic, GABAergic, serotonergic, and cholinergic pathways would also help clarify whether the observed effects are related to H1 receptor modulation or broader central nervous system activity. These additional investigations are necessary before considering translational or clinical relevance.^[29]

CONCLUSION

The present study provides preliminary evidence that selected antihistaminic drugs exhibit anxiolytic-like activity in the Elevated Plus Maze model. Promethazine produced a stronger behavioural response than cetirizine, while diazepam showed the highest response among the tested groups. The preservation of total arm entries suggests that the observed changes were not mainly attributable to gross locomotor suppression. Overall, these hypothesis-generating findings warrant further investigation of antihistaminic agents as potential candidates for drug repurposing in anxiety-related conditions, while emphasizing the need for additional behavioural and mechanistic validation before clinical translation.

Declarations

Ethics Approval

All experimental procedures involving animals were conducted in accordance with applicable institutional guidelines for the care and use of laboratory animals. The

study protocol was reviewed and approved by the Institutional Animal Ethics Committee, Approval No. 688/2/C-CPCSEA/SM/20260314.

Conflicts of Interest

The authors declare that they have no conflicts of interest relevant to this study.

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Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

- **Shanmugapriya M:** Conceptualization, Study Design, Methodology, Manuscript Writing, Review and Editing, Final Manuscript Approval.
- **Sivakumar G M:** Supervision, Methodology, Data Analysis, Interpretation of Results, Manuscript Review.
- **Perumal P:** Supervision, Scientific Guidance, and Final Manuscript Approval.
- **Abishek R K:** Experimental Work, Data Collection, Data Analysis, and Manuscript Preparation.
- **Arunraj K:** Experimental Work, Data Collection, Data Analysis, and Manuscript Preparation.
- **Gobika R:** Experimental Work, Data Collection, Data Analysis, and Manuscript Preparation.
- **Shaveetha A:** Experimental Work, Data Collection, Data Analysis, and Manuscript Preparation.
- **Shibi S R:** Experimental Work, Data Collection, Data Analysis, and Manuscript Preparation.

All authors have read and approved the final manuscript.

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