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Neuroprotective effects of pyridoxine–levetiracetam on cognitive dysfunction and oxidative stress in PTZ -kindled rats

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Abstract:
Pentylenetetrazole-induced kindling causes progressive seizures, behavioural impairment and oxidative stress. Therefore, it is of interest to evaluate the effect of pyridoxine combined with low-dose levetiracetam in PTZ-kindled Wistar rats. The combination of pyridoxine 200 mg/kg and levetiracetam 50 mg/kg significantly reduced seizure severity, escape latency and malondialdehyde levels compared with PTZ controls ($P < 0.0001$). Combination therapy significantly increased glutathione, superoxide dismutase and catalase activities and produced the lowest median modified Racine score. Pyridoxine therefore potentiated the anticonvulsant and neuroprotective effects of low-dose levetiracetam during epileptogenesis.

Keywords: pyridoxine; levetiracetam; pentylenetetrazol (PTZ) kindling; oxidative stress; cognition; epilepsy; rats

Background:
Epilepsy is a chronic neurological disorder marked by recurrent unprovoked seizures and substantial cognitive, behavioural, psychosocial and disability burdens worldwide, especially in low- and middle-income settings with limited access to diagnosis and treatment **[1].** Although antiseizure medications are effective for many patients, a clinically important proportion continues to experience uncontrolled seizures or treatment-related adverse effects **[2].** Therefore, interventions that not only suppress seizures but also limit seizure-associated neurobehavioral impairment remain relevant therapeutic targets. Recurrent seizures in epilepsy contribute to cognitive dysfunction by disrupting attention, learning, memory and executive function through neuronal hyperexcitability, altered synaptic plasticity, neuroinflammation and oxidative stress **[3]**. In addition, some antiseizure medications may contribute to cognitive or behavioural adverse effects, depending on dose, treatment duration, individual susceptibility and concurrent neurological disease **[4]**. Consequently, experimental studies of epilepsy should ideally assess functional outcomes in addition to seizure severity, particularly when evaluating adjunctive interventions with potential neuroprotective actions **[5].** The Pentylenetetrazole (PTZ) kindling model is widely used to study progressive seizure susceptibility and epileptogenesis. Repeated administration of sub convulsive PTZ doses produces escalating seizure responses that resemble the gradual development of a hyperexcitable neuronal network **[6]**. Beyond overt convulsive activity, PTZ kindling has been associated with behavioural alterations, learning and memory impairment and biochemical evidence of oxidative stress **[7]**. Lipid peroxidation and depletion of endogenous antioxidant systems may contribute to neuronal vulnerability during recurrent seizures, making oxidative-stress markers such as malondialdehyde, reduced glutathione, superoxide dismutase and catalase biologically relevant outcome measures in this model **[8, 9].** Levetiracetam is a commonly used antiseizure medication with a distinct pharmacological profile. Its principal molecular target is synaptic vesicle protein 2A, a vesicle-associated protein involved in neurotransmitter release and synaptic function **[10]**. Through modulation of SV2A-related synaptic activity, levetiracetam reduces neuronal hyperexcitability and provides broad antiseizure efficacy **[11]**. However, despite its favorable pharmacokinetic profile and relatively limited drug interaction potential, levetiracetam may be associated with neuropsychiatric and behavioural adverse effects in susceptible individuals **[12]**. These concerns support investigation of safe adjunctive strategies that may preserve seizure control while improving neurobehavioral and biochemical outcomes. Pyridoxine, or vitamin B6, is a precursor of pyridoxal-5′-phosphate, an essential cofactor for several enzymes involved in neurotransmitter metabolism **[13]**. Of particular relevance to epilepsy, pyridoxal-5′-phosphate is required for glutamate decarboxylase-mediated synthesis of gamma-aminobutyric acid, the major inhibitory neurotransmitter in the central nervous system **[14].** Disturbances in vitamin B6 availability or metabolism can reduce inhibitory neurotransmission and increase seizure susceptibility, while pyridoxine-responsive seizure disorders provide clinical evidence for the importance of this pathway **[15, 16]**. Experimental evidence also suggests that pyridoxine influences hippocampal enzymes involved in gamma-aminobutyric acid synthesis and degradation, providing a biological rationale for examining its role in seizure-associated neurobehavioral dysfunction **[17].** The potential interaction between pyridoxine and levetiracetam is pharmacologically relevant, as pyridoxine supports inhibitory neurotransmitter synthesis and antioxidant defence, whereas levetiracetam modulates presynaptic neurotransmission via synaptic vesicle protein 2A (SV2A) **[18]**. The distinct yet complementary mechanisms of pyridoxine and levetiracetam provide a pharmacological rationale for combined therapy in seizure-associated neuronal dysfunction, although current clinical evidence for pyridoxine in reducing levetiracetam-related behavioural adverse effects is insufficient to support definitive therapeutic recommendations **[19]**. Therefore, it is of interest to evaluate whether pyridoxine 200 mg/kg, administered alone or in combination with low-dose levetiracetam 50 mg/kg, improves cognitive performance and oxidative-stress markers in PTZ-kindled rats.

Materials and Methods:
Study design:

This experimental animal study evaluated the effect of pyridoxine 200 mg/kg, alone and in combination with low-dose levetiracetam 50 mg/kg, on cognitive/behavioural performance, seizure progression and oxidative-stress markers in a pentylenetetrazol (PTZ)-kindling model. The study was designed and reported in accordance with the ARRIVE 2.0 guidelines for animal research reporting **[20].**

Four treatment groups were included:
[1] PTZ control
[2] Pyridoxine 200 mg/kg + PTZ
[3] Levetiracetam 50 mg/kg + PTZ
[4] Pyridoxine 200 mg/kg + levetiracetam 50 mg/kg + PTZ
Each group comprised six rats, with a total sample size of 24 rats (**Table 1**).

Animals and ethical considerations:
Adult male Wistar rats aged 6–8 weeks and weighing 180–220 g was used. Animals were procured from rodent research India Pvt. Ltd. (CCSEA approved facility), Dist.-Jind and Haryana and acclimatized for at least 7 days before the start of experimentation in Central Animal Facility, SGT University, Gurugram, Haryana. Rats were housed in polypropylene cages under standard laboratory conditions with controlled temperature, relative humidity and a 12-hour light/dark cycle. Standard pellet diet and water were provided ad libitum. All procedures were undertaken after approval from the Institutional Animal Ethics Committee, under approval number-SGTU/IAEC/2024/02. Animal care and experimental handling were performed in accordance with the Guide for the Care and Use of Laboratory Animals and applicable institutional regulations **[21].**

Drugs and chemicals:
Pentylenetetrazole, pyridoxine hydrochloride and levetiracetam were procured in powdered form from Sun Pharmaceutical Laboratories Ltd. Gurgaon, Haryana & Central Drug House (P) Ltd., Delhi. PTZ was freshly prepared in normal saline immediately before administration. Pyridoxine and levetiracetam were prepared in normal saline. Drug doses were calculated according to individual body weight and administered in a fixed volume of 1mL/100 g body weight respectively.

Experimental groups and treatment schedule:
Pyridoxine 200 mg/kg and levetiracetam 50 mg/kg were administered orally before PTZ administration. Animals in the PTZ control group received the corresponding group. PTZ was administered at 35 mg/kg by intraperitoneally according to the scheduled kindling protocol for 29 days. Repeated administration of sub convulsive PTZ doses is an established model for progressive seizure susceptibility and epileptogenesis **[22].**

PTZ-kindling procedure and seizure assessment:
Following PTZ administration, individual rats were placed in transparent observation chambers and monitored for 30 minutes. Seizure severity was graded using the modified Racine scale **(Table 2)**. Serial seizure scores were recorded from day 1 to day 29. Daily Racine score, cumulative seizure score and final-day seizure severity were recorded as seizure-related outcome measures. The Racine scale is a standard behavioural grading method for motor seizure severity in experimental epilepsy studies **[23].**

Cognitive and behavioural assessment:
Cognitive and behavioural assessment was performed on days 14, 30 and 36 using the Morris Water Maze. Behavioural testing was performed in a quiet room under uniform environmental conditions, including consistent lighting and timing. Animals were allowed to acclimatize to the testing environment before assessment.

Brain tissue collection and homogenate preparation:
After completion of behavioural assessment, rats were euthanized using carbon dioxide (CO_2) inhalation in a dedicated euthanasia chamber. The brain was rapidly removed, washed with ice-cold normal saline and dissected to obtain hippocampus. Tissue samples were homogenized in 0.1 M phosphate buffer (pH 7.4) under cold conditions. The homogenate was centrifuged at 6000 rpm for 30 minutes at 4°C. The resulting supernatant was collected and used for biochemical estimation.

Estimation of oxidative-stress markers:
Malondialdehyde (MDA) was estimated as an indicator of lipid peroxidation using the thiobarbituric acid reaction method described by Diaz De Leon & Borges **[24].** Results were expressed as nmol MDA/mg protein. Reduced glutathione (GSH) was estimated using the 5, 5′-dithiobis-(2-nitrobenzoic acid) method described by Ellman **[25]**. Results were expressed as µmol GSH/mg protein. Superoxide dismutase (SOD) activity was estimated using the epinephrine auto-oxidation method described by Rosa *et al.* **[26]**. Results were expressed as U/mg. Catalase activity was estimated by measuring the decomposition of hydrogen peroxide using the method described by de Melo *et al.* **[27]**. Results were expressed as U/mg protein.

Outcome measures:
The primary outcomes were escape latencies at days 14, 30 and 36, along with brain MDA, GSH, SOD and catalase levels. Secondary outcomes were daily seizure score, cumulative seizure burden and final modified Racine seizure score.

Statistical analysis:
Data were analysed using Graph Pad Prism version 8.0.2. The Shapiro–Wilk test was used to assess normality of continuous data. Normally distributed data were presented as mean ± standard error of the mean and compared using one-way analysis of variance followed by Tukey's multiple-comparison test. Non-normally distributed data were presented as median with minimum and maximum values and compared using the Kruskal–Wallis test followed by Dunn's post-hoc test. Longitudinal seizure scores and repeated cognitive/behavioural

observations were analysed using two-way repeated-measures analysis of variance or a mixed-effects model, with treatment group and study day included as fixed effects. Where a significant group × time interaction was observed, Tukey's multiple-comparison test was used for pairwise analysis. A two-sided P value <0.05 was considered statistically significant.

Table 1: Animals were allocated into four treatment groups as shown below

Group	Treatment	Number of rats
I	PTZ control	6
II	Pyridoxine 200 mg/kg + PTZ	6
III	Levetiracetam 50 mg/kg + PTZ	6
IV	Pyridoxine 200 mg/kg + levetiracetam 50 mg/kg + PTZ	6

Table 2: Modified racine scale for assessment of seizure severity in rats

Score	Seizure manifestation
0	No seizure activity
1	Jaw clonus, ear or facial twitching
2	Myoclonic body jerks
3	Forelimb clonus
4	Generalised tonic-clonic seizures
5	Hindlimb tonic extension

Table 3: Effect of pyridoxine 200 mg/kg, levetiracetam 50 mg/kg and their combination on escape latency at days 14, 30 and 36 in PTZ-kindled rats

Treatment group	Escape latency on day 14 (s)	Escape latency on day 30 (s)	Escape latency on day 36 (s)
PTZ control	35.57 ± 0.75	49.03 ± 0.91	56.57 ± 0.87
Levetiracetam 50 mg/kg + PTZ	22.05 ± 0.59[a]	19.15 ± 0.55[a]	17.37 ± 0.54[a]
Pyridoxine 200 mg/kg + PTZ	19.12 ± 0.63[ab]	14.45 ± 0.55[ab]	12.15 ± 0.48[ab]
Pyridoxine 200 mg/kg + levetiracetam 50 mg/kg + PTZ	16.43 ± 0.58[abc]	10.08 ± 0.52[abc]	7.58 ± 0.48[abc]
Overall P value	<0.0001	<0.0001	<0.0001

Values are expressed as mean ± SEM; n = 6/group. Lower escape latency indicates better learning and memory performance.
One-way ANOVA followed by Tukey's multiple-comparison test.
[a]P < 0.0001 versus PTZ control.
[b]P < 0.05 versus levetiracetam 50 mg/kg + PTZ
[c]P < 0.05 versus pyridoxine 200 mg/kg + PTZ

Table 4: Effect of pyridoxine 200 mg/kg, levetiracetam 50 mg/kg and their combination on brain oxidative-stress markers in PTZ-kindled rats

Treatment group	MDA (nmol/mg protein)	GSH (µmol/mg protein)	SOD (U/mg protein)	Catalase (U/mg protein)
PTZ control	5.02 ± 0.10	18.18 ± 0.52	4.13 ± 0.18	11.88 ± 0.54
Levetiracetam 50 mg/kg + PTZ	2.31 ± 0.07[a]	39.20 ± 0.89[a]	10.73 ± 0.31[a]	28.02 ± 0.74[a]
Pyridoxine 200 mg/kg + PTZ	2.02 ± 0.07[ab]	41.80 ± 0.75[a]	11.63 ± 0.24[a]	30.17 ± 0.58[a]
Pyridoxine 200 mg/kg + levetiracetam 50 mg/kg + PTZ	1.40 ± 0.05[abc]	45.78 ± 0.54[abc]	13.08 ± 0.30[abc]	33.00 ± 0.53[abc]
Overall test statistic	F(3,20) = 477.14	F(3,20) = 318.12	F(3,20) = 231.72	F(3,20) = 247.07
Overall P value	<0.0001	<0.0001	<0.0001	<0.0001

Values are expressed as mean ± SEM; n = 6/group. One-way ANOVA followed by Tukey's multiple-comparison test.
[a]P < 0.0001 versus PTZ control.
[b]P < 0.05 versus levetiracetam 50 mg/kg + PTZ
[c]P < 0.05 versus pyridoxine 200 mg/kg + PTZ

Table 5: Effect of pyridoxine 200 mg/kg, levetiracetam 50 mg/kg and their combination on cumulative seizure burden and day-29 modified Racine score in PTZ-kindled rats

Treatment group	Cumulative seizure score	Day-29 modified Racine score
PTZ control	51.50 ± 0.85	5.0 (5.0–5.0)
Levetiracetam 50 mg/kg + PTZ	21.00 ± 0.45[a]	2.0 (2.0–3.0)
Pyridoxine 200 mg/kg + PTZ	15.50 ± 0.56[ab]	2.0 (2.0–2.0)
Pyridoxine 200 mg/kg + levetiracetam 50 mg/kg + PTZ	9.00 ± 0.52[abc]	1.0 (1.0–2.0)
Overall test statistic	F(3,20) = 944.22	H = 20.51
Overall P value	<0.0001	0.0001

Cumulative seizure score is expressed as mean ± SEM; n = 6/group; one-way ANOVA followed by Tukey's multiple-comparison test.
Day-29 modified Racine score is expressed as median (minimum–maximum); Kruskal–Wallis test.
[a]P < 0.0001 versus PTZ control.
[b]P < 0.0001 versus levetiracetam 50 mg/kg + PTZ
[c]P < 0.0001 versus pyridoxine 200 mg/kg + PTZ

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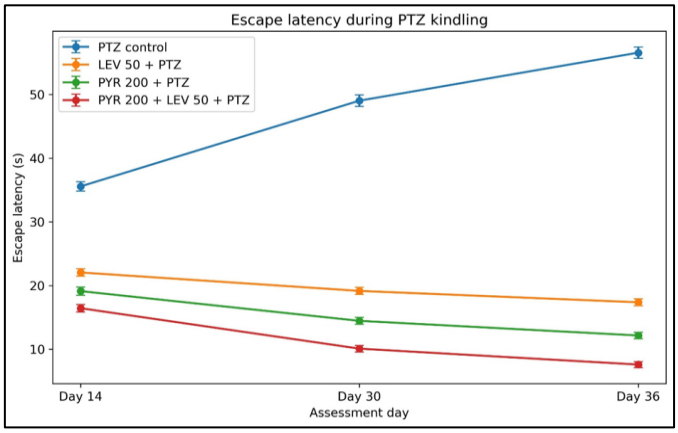

Figure 1: Effect of pyridoxine 200 mg/kg, levetiracetam 50 mg/kg and their combination on escape latency at days 14, 30 and 36 in PTZ-kindled rats. Values are expressed as mean ± SEM; n = 6/group.

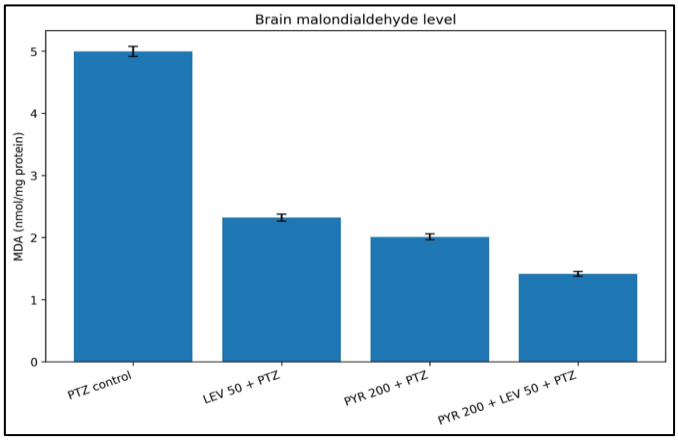

Figure 2: Effect of treatment on brain malondialdehyde concentration in PTZ-kindled rats. Values are expressed as mean ± SEM; n = 6/group.

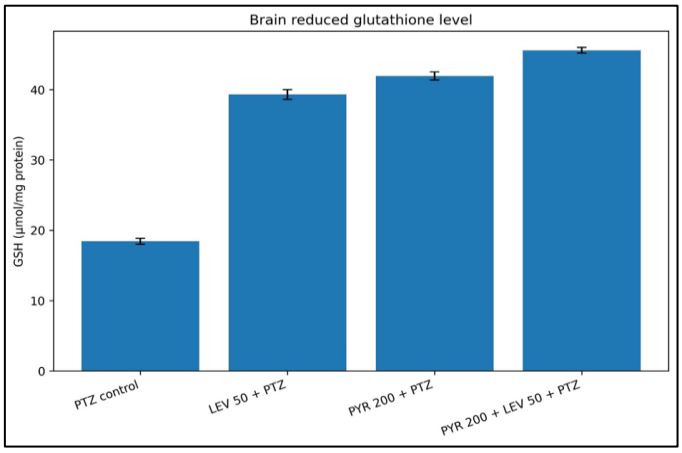

Figure 3: Effect of treatment on brain reduced glutathione concentration in PTZ-kindled rats. Values are expressed as mean ± SEM; n = 6/group.

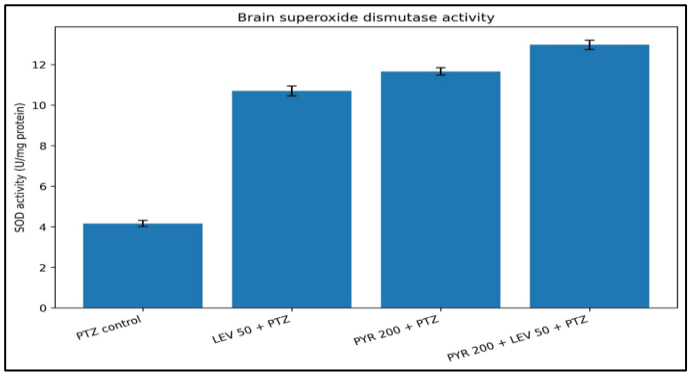

Figure 4: Effect of treatment on brain superoxide dismutase activity in PTZ-kindled rats. Values are expressed as mean ± SEM; n = 6/group.

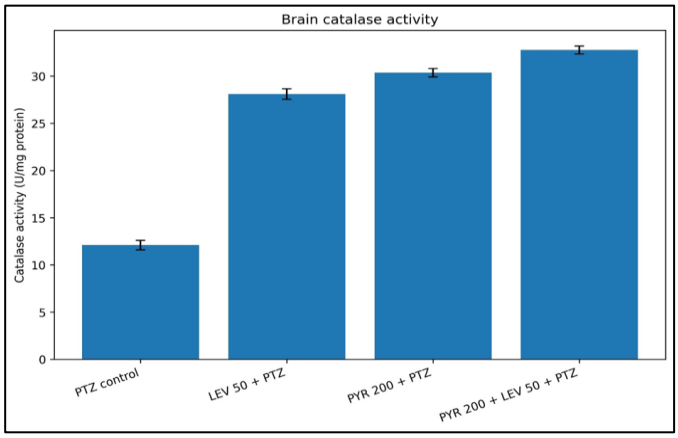

Figure 5: Effect of treatment on brain catalase activity in PTZ-kindled rats. Values are expressed as mean ± SEM; n = 6/group.

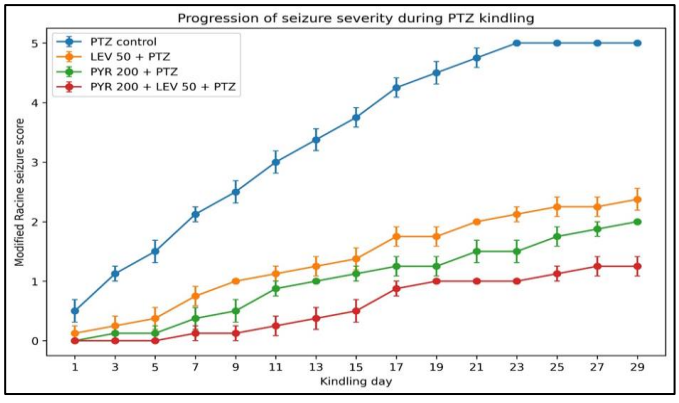

Figure 6: Serial modified Racine seizure scores during PTZ kindling among the selected treatment groups. Values are expressed as mean ± SEM; n = 6/group.

Results:

Escape latency differed significantly among the four treatment groups on days 14, 30 and 36 **(Table 1; Figure 1)**. The PTZ control group expressed progressive prolongation of escape latency from 35.57 ± 0.75 s on day 14 to 56.57 ± 0.87 s on day 36.

In contrast, all active-treatment groups had significantly lower escape latency than the PTZ control group at each assessment point. The pyridoxine 200 mg/kg plus levetiracetam 50 mg/kg group showed the shortest escape latency on day 14 (16.43 ± 0.58 s), day 30 (10.08 ± 0.52 s) and day 36 (7.58 ± 0.48 s). At day 36, the combination group had lower escape latency than both pyridoxines alone (12.15 ± 0.48 s) and levetiracetam alone (17.37 ± 0.54 s) **(Table 3, Figure 1).** Significant between-group differences were observed for MDA, GSH, SOD and catalase levels **(Table 4, Figures 2–5).** PTZ-treated rats showed the highest MDA concentration and the lowest GSH concentration, SOD activity and catalase activity. The bination group had the lowest MDA concentration (1.40 ± 0.05 nmol/mg protein), which was significantly lower than the PTZ control, levetiracetam-alone and pyridoxine-alone groups **(Table 4, Figure 2).** The combination also showed the highest GSH concentration (45.78 ± 0.54 µmol/mg protein), SOD activity (13.08 ± 0.30 U/mg protein) and catalase activity (33.00 ± 0.53 U/mg protein) **(Table 4, Figures 3–5).** Compared with levetiracetam alone, pyridoxine alone significantly reduced MDA concentration but did not significantly alter GSH, SOD, or catalase values. Addition of pyridoxine to levetiracetam significantly improved all four oxidative-stress markers compared with either monotherapy **(Table 4).** PTZ control rats showed progressive seizure escalation throughout the kindling period, reaching a modified Racine score of 5 in all animals by day 29 **(Table 5, Figure 6).** All active-treatment groups showed lower cumulative seizure scores than the PTZ control group. The cumulative seizure score was lowest in the pyridoxine–levetiracetam combination group (9.00 ± 0.52), followed by pyridoxine alone (15.50 ± 0.56) and levetiracetam alone (21.00 ± 0.45). Cumulative seizure score differed significantly among the four groups and all pairwise comparisons were significant on Tukey's multiple-comparison test **(Table 5).** The median day-29 modified Racine score was 5.0 in the PTZ control group, 2.0 in the levetiracetam-alone and pyridoxine-alone groups and 1.0 in the combination group **(Table 5).** The serial seizure-score trajectory showed persistent attenuation of seizure severity in the combination group throughout the study period **(Figure 6).**

Discussion:

The present study evaluated the effect of pyridoxine 200 mg/kg, levetiracetam 50 mg/kg and their combination on seizure progression, cognitive/behavioural outcome and oxidative-stress markers in PTZ-kindled rats. The principal finding was that the pyridoxine–levetiracetam combination produced the most favourable overall response. Compared with the PTZ control group, the combination group showed lower recorded cognitive/behavioural scores at days 14, 30 and 36, lower cumulative seizure burden and final Racine score, lower MDA concentration and higher GSH, SOD and catalase activities. The PTZ control group showed progressive seizure escalation, with all rats reaching a modified Racine score of 5 by day 29. This pattern indicates successful kindling development and is consistent with the recognised ability of repeated sub convulsive PTZ exposure to produce progressive seizure susceptibility and epilepsy-associated behavioural comorbidities **[22, 28].** In the present study, the cumulative seizure score was markedly lower in the pyridoxine–levetiracetam combination group than in the PTZ control group. The lower terminal Racine score in the combination group further indicates that pyridoxine may augment the protective effect of low-dose levetiracetam during PTZ-induced epileptogenesis. The observed response to pyridoxine has a plausible neurochemical basis. Pyridoxine is converted to pyridoxal-5′-phosphate, an essential cofactor for glutamate decarboxylase-mediated synthesis of gamma-aminobutyric acid. Thus, pyridoxine availability may support inhibitory neurotransmission during recurrent neuronal hyperexcitability **[14, 17]**. Earlier experimental work by Sharma *et al.* showed that pyridoxine-deficient rats exhibited enhanced susceptibility to picrotoxin- and PTZ-induced seizures, supporting the association between vitamin B6 status and seizure threshold **[31]**. Therefore, the reduction in seizure burden observed with pyridoxine-containing treatment groups may be related, at least partly, to improved GABAergic inhibitory capacity. The present findings should, however, be interpreted in the context of the complex and timing-dependent effects of B vitamins in seizure models. Aleshin *et al.* reported that the physiological and biochemical consequences of PTZ-induced seizures, as well as the response to vitamins B1 and B6, varied according to sex, treatment schedule and seizure protocol **[32]**. In a subsequent PTZ-kindling study, the same group reported that vitamin administration influenced metabolic and behavioural correlations after seizures, but did not uniformly alter seizure measures under all experimental conditions **[33]**. These observations suggest that the response to pyridoxine is likely influenced by dose, timing of administration, type of seizure model, duration of treatment and concurrent pharmacotherapy. The present protocol differs because pyridoxine 200 mg/kg was administered in a repeated PTZ-kindling paradigm and evaluated in combination with levetiracetam. Levetiracetam alone reduced cumulative seizure burden and improved oxidative-stress markers compared with the PTZ control group. This is consistent with its established action through synaptic vesicle protein 2A modulation and reduction of abnormal neuronal hyperexcitability **[10, 11].** However, the pyridoxine–levetiracetam combination showed lower seizure scores and a more favourable oxidative-stress profile than levetiracetam 50 mg/kg alone. Pyridoxine may support inhibitory neurotransmitter synthesis and cellular redox homeostasis, whereas levetiracetam predominantly modifies presynaptic neurotransmitter release. The two interventions may therefore act through complementary mechanisms. Nevertheless, a formal pharmacodynamic interaction analysis was not performed; thus, the observed response should be described as enhanced combination efficacy rather than proven pharmacological synergy. The present findings are comparable with previous studies in which levetiracetam was combined with neuroprotective or antioxidant adjuncts. Löscher and White reported that levetiracetam combined with liraglutide reduced PTZ-kindling development and behavioural comorbidities in mice, accompanied by improvement in brain antioxidant status

and brain-derived neurotrophic factor expression **[29]**. Similarly, Javaid *et al.* showed that levetiracetam combined with sodium selenite prevented PTZ-induced kindling and improved behavioural abnormalities in rats **[30].** Although pyridoxine differs pharmacologically from liraglutide and sodium selenite, these studies support the principle that adjunctive agents with antioxidant or neuroprotective properties may improve outcomes beyond those achieved by antiseizure therapy alone. Oxidative stress was markedly altered in the PTZ control group, which showed the highest MDA concentration and the lowest GSH, SOD and catalase values **(Table 3, Figures 2–5).** This pattern is consistent with previous evidence that PTZ kindling is associated with increased lipid peroxidation, reduced endogenous antioxidant activity, mitochondrial dysfunction and neuronal injury **[8, 9 and 28]**. In the present study, pyridoxine and levetiracetam monotherapy each partially improved the oxidative-stress profile. However, the combination group showed the lowest MDA level and the highest GSH, SOD and catalase activities, indicating the most favourable redox status **(Table 3).** The biochemical findings are consistent with previous PTZ-kindling studies in which interventions that reduced lipid peroxidation and restored antioxidant defences were associated with lower seizure severity and improvement in behavioural outcomes. Azim *et al.* reported that agomelatine reduced PTZ-kindling severity, oxidative stress and behavioural despair in mice **[34]**. Kumar *et al.* showed that lycopene combined with sodium valproate improved PTZ-kindling scores and restored antioxidant markers, including GSH, SOD, catalase and lipid-peroxidation indices **[35]**. Although the active interventions differed from those used in the present study, the direction of response was similar: attenuation of oxidative stress was associated with reduced seizure progression. The combination group also showed the most favourable recorded cognitive/behavioural score profile at each assessment time point. PTZ kindling is associated with behavioural and cognitive disturbances, including abnormalities in learning, memory, exploratory behaviour, anxiety-like behaviour and depressive-like responses **[28, 34].** The progressive increase in score in the PTZ control group and lower scores in the treatment groups suggest that recurrent seizures were associated with worsening behavioral performance, whereas pyridoxine-containing treatment may have limited this deterioration. The present findings have potential translational relevance because pyridoxine is inexpensive, widely available and may complement levetiracetam through mechanisms distinct from SV2A modulation. The results suggest that pyridoxine may be a useful neuroprotective adjunct during epileptogenesis, particularly when low-dose levetiracetam is used. However, the present findings are preclinical and cannot be directly extrapolated to patients with epilepsy. Several limitations should be considered. First, only male Wistar rats were studied; therefore, sex-specific treatment effects could not be evaluated. Second, the sample size was modest, although statistically significant between-group differences were observed. Third, MDA, GSH, SOD and catalase are indirect markers of oxidative stress and do not establish the complete molecular pathway responsible for neuroprotection. Fourth, histopathology, inflammatory mediators, mitochondrial markers, pyridoxal-5′-phosphate concentrations, GABA levels and neurotransmitter-release markers were not assessed.

Conclusion:
Pyridoxine 200 mg/kg combined with levetiracetam 50 mg/kg produced the most favourable seizure, oxidative-stress and cognitive/behavioural score profile in PTZ-kindled rats. The combination reduced seizure severity and cumulative seizure burden, lowered lipid peroxidation, restored endogenous antioxidant activity and showed favourable longitudinal behavioural outcomes. These data support further mechanistic, histopathological and pharmacological-interaction studies of pyridoxine as a potential adjunct to levetiracetam in experimental epileptogenesis.

Conflict of interest:
The authors declare that they have no conflict of interest.

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Abbreviations:
ANOVA: Analysis of variance
ARRIVE: Animal Research: Reporting of *In Vivo* Experiments
GABA: Gamma-aminobutyric acid
GSH: Reduced glutathione
IAEC: Institutional Animal Ethics Committee
MDA: Malondialdehyde
PTZ: Pentylenetetrazole
SEM: Standard error of the mean
SOD: Superoxide dismutase
SV2A: Synaptic vesicle protein 2A

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