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Evaluation of PTZ-induced Seizures in Different Animal Models

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Abstract

Epilepsy is a chronic neurological disorder characterized by recurrent seizures resulting from abnormal neuronal excitability. The present study investigates the induction and evaluation of seizures using Pentylenetetrazole (PTZ) across four animal models: Swiss albino mice, Wistar rats, Golden Syrian hamsters, and zebra fish. PTZ, a GABAA receptor antagonist, was administered intraperitoneally in species-specific doses to trigger seizure activity. Seizure characteristics were recorded using standardized behavioral parameters such as latency period, clonic and tonic seizures, and postictal depression. Zebrafish behavior was assessed using a time-budget scoring system for epileptic responses. Results demonstrated species-specific variations in seizure onset, severity, recovery, and mortality, with hamsters exhibiting the shortest latency of 32.1 ± 18.38 s and the highest variability in seizure phases. Histopathological examination of the mice brain tissue post-seizure revealed normal hippocampal and cortical structures, indicating limited neurodegeneration under the tested conditions. The findings underscore the utility of multiple animal models in seizure research and support the therapeutic potential of herbal and chemical compounds in managing epilepsy.

Keywords: Epilepsy, Pentylenetetrazole (PTZ), Mice, Rat, Hamster, Zebrafish, Seizure.

Introduction

Epilepsy is a common neurological disorder that is associated with changes in psychological, emotional, and educational factors. More than half of individuals with epilepsy experience some form of cognitive impairment, often accompanied by abnormal behavioral symptoms (Gupta *et al*., 2003). As a neurodegenerative condition, epileptogenesis may be linked to the production of oxidative species. The brain is particularly vulnerable to these reactive oxygen species because it requires a constant and high supply of oxygen and energy for metabolic processes. During seizure activity, the increased demand for metabolic energy can disrupt the oxidative balance within the central nervous system, contributing to neuronal damage (Mishchenko *et al.,* 2022).

Although the genetic basis of common forms of epilepsy in humans remains unclear, research has identified several genes responsible for rarer, monogenic forms of the disorder. These discoveries have largely been made through linkage analysis, association studies, and positional cloning. Additionally, studying epilepsy in animal models, especially mice, has helped researchers identify candidate genes that may also play a role in human epilepsy (Meisler *et al.,* 2001; Escayg *et al.*, 2000).

Gamma-aminobutyric acid (GABA) plays a crucial role in regulating neuronal excitability in the central nervous system through two main receptor types: GABAA and GABAB. GABAA receptors function as ligand-gated ion channels, while GABAB receptors are G-protein-

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coupled receptors that mediate slower inhibitory responses by increasing potassium conductance and reducing calcium influx. These mechanisms contribute to the generation of inhibitory presynaptic potentials. However, alterations in GABAergic signaling can disrupt this balance, potentially contributing to epileptogenesis. In particular, changes in inhibitory control may enhance excitatory postsynaptic activity and promote the synchronization of neuronal burst discharges, which are characteristic of seizure activity (Yin *et al.,* 2013).

Among experimental approaches, two of the most widely used animal models for studying seizures are subcutaneous pentylenetetrazol (s.c.-PTZ)-induced seizures and maximal electroshock (MES)-induced seizures in mice (Baruzzi et al., 1995). The PTZ-induced seizure model, originally described by Mason and Cooper in rats (1972), remains a standard method for screening anticonvulsant drugs. Commonly used animal models for PTZ-induced seizures include mice, rats, and zebrafish.

A wide range of animal models has been developed to better understand epileptogenesis, discover new antiepileptic drugs (ASDs), and investigate their mechanisms of action (Rubio *et al.,* 2012). In PTZ-based studies, different mouse strains show varying levels of sensitivity. For example, Swiss albino and BALB/c mice are generally more sensitive to PTZ, whereas C57BL/6 mice tend to be more resistant. In this study, Swiss albino mice were selected due to their higher susceptibility. Age is another important factor, as younger mice are typically more prone to PTZ-induced seizures compared to older ones (Shimada *et al.,* 2018; Nokubo *et al.,* 1986). Notably, PTZ and electrical kindling models offer advantages such as low mortality rates and the successful induction of epilepsy in over 80% of animals (Bertram, 2007; Welzel *et al.*, 2019).

In addition to rodents, zebrafish have emerged as a valuable model in epilepsy research. Their small size, ease of maintenance, low cost, high reproductive rate, and rapid external development make them especially useful for large-scale screening of compounds with potential anticonvulsant properties (Avdesh *et al.,* 2012).

The objective of the present study is to comparatively assess the characteristics of pentylenetetrazol (PTZ)-induced seizures in mice, rats, hamsters, and zebrafish.

MATERIALS AND METHODS

Animals with Ethical Statement:

The animal experiment was carried out as per the instructions in the protocol number MB/IAEC/2024/02/02, MB/IAEC/2024/01/04, 05 given by the ethical committee of Mass Biotech, Chengalpattu, with CPCSEA registration number 2084/PO/RcBiBt/S/19/CPCSEA. The study was conducted at the duration of June 2024 to November 2024. Swiss albino mice (male) of weight 40±5 g, Wistar rat of weight 150±30 g, and hamster of weight 80±20 g were housed in polypropylene cages with stainless steel top grills, and corn cob was used as bedding material. All animals were kept in the animal room with the following environmental conditions; 23±3°C temperature, relative humidity at 50±20%, photoperiod of 12hr light/12hr dark, and the sound level was kept below 65 dB. Individual Polypropylene cages were used to house the animals with corn cob bedding, and they had ad libitum access to water (RO water) and a commercially available Rodent pellet diet with 18 percent protein. Zebrafish were kept in a circulating system that continuously filters and aerates the system water to maintain the water quality required for a healthy aquatic environment. The conditions were ensured as per CPCSEA regulations.

Experimental protocol for seizure induction:

Swiss Albino mice, Wistar rat, and Golden Syrian hamster were used for the study. PTZ is used for the induction of seizures. PTZ was purchased from SRL Chemicals. It was dissolved in 0.9% saline, and based on the body weight of animals, PTZ was administered intraperitoneally (IP) to each animal (Table 1). Every animal was examined individually after administering PTZ for 7 min.

PTZ-induced seizures in Zebrafish:

To induce epilepsy in wild-type zebrafish, PTZ (170 mg/kg body weight, intraperitoneal) was administered. The fish was carefully transferred to a container containing ice water maintained at 17 °C, the fish exhibited reduced swimming activity and eventually became immobile for short time. The fish was then immediately transferred to a sponge trough and positioned with the abdomen facing upward and the gills supported within the trough.

Using a Hamilton syringe, 5–10 µL of PTZ solution (prepared in 0.9% saline) was rapidly injected into the peritoneal cavity.IP administration in zebrafish may be inconsistent, and mortality does not always occur during seizure experiments The injection site was located along the midline, cranial to the base of the pelvic fin (Stewart *et al.*, 2011). Following injection, the zebrafish was placed in a separate container for further observation and evaluation.

Seizure recording for mice, rat, and hamster:

The seizure recording was based on these parameters:

1. Latency period (Time between administration of PTZ and first jerk) (Singh *et al.*, 2011),
2. Clonic period (period of repeated clonic jerks of the forelimbs and hindlimbs with loss of the righting reflex) (Kondziella *et al.*, 2002),
3. Tonic period (falling on the side, followed by forelimb and hindlimb tonic contractions) (Zolkowska *et al*., 2012),
4. Postictal depression (the period between the end of tonic seizure to the normal stage of mice).

The end of postictal depression indicates the end of a full cycle of seizures. After the administration of PTZ, the convulsive activity of the animal was recorded for 7 minutes and used in further interpretations. The evaluation is based on only two parameters, i.e., Latency period and Seizure period (combination of clonic period, tonic period, and postictal depression) (Hakimi Naeini *et al*., 2024).

After the evaluation, the Samples were randomly collected, considering only mice. animal was using isoflurane sacrificed, and the brain was collected and stored in 10% Formalin for Histopathological analysis by using H and E staining.

Seizure activity was monitored, and animals were euthanized or removed from the experiment if seizures exceeded the predetermined humane endpoint duration.

Seizure recording for Zebrafish:

For Zebrafish seizure recording, the time budget method was used to record the movements. For every 30 seconds, the zebrafish will be given a behavioral score. Epileptic behaviors of zebrafish are listed below(Watters *et al*., 2009; Harms *et al*., 2011).

0. Short swim mainly at the bottom of the tank (Normal)
1. Increased swimming activity and high frequency of opercular movements in an inclined position.
2. Burst swimming of left & right movements.
3. Swimming in a circular motion.
4. Clonic seizure-like behavior (abnormal body rhythm).

5. Tonic seizure-like behavior (Falling to the bottom of the tank with loss of body posture).
6. Postictal Depression (Normal breathing with no movement, staying in one place)
7. Death(Mussulini *et al.*, 2013).

RESULT:

For seizure evaluation, PTZ was given to the animals (n=8), and each animal had a seizure recorded for 7 minutes for the highest metabolic rate of neural recovery in rodents . The seizure was evaluated as the Latency Period and Seizure Period.

This table compares seizure characteristics across three species: mice, rats, and hamsters. It presents the latency period, duration of clonic and tonic seizures, postictal depression, and mortality rates of death percent.

These data indicate species-specific differences in seizure onset, duration, and severity, with hamsters exhibiting the shortest latency and clonic seizure duration, while mice showed the longest postictal depression period.

The results show that the latency period before seizure onset varies, with some subjects experiencing seizures sooner than others. Clonic seizure durations exhibit a wide range, indicating variability in the intensity of this seizure phase. Tonic seizures tend to last for a moderate duration, while postictal depression periods differ substantially, reflecting varied recovery times after seizures. Mortality rates remain relatively low, suggesting that most subjects survive the induced seizures.

In mice, rat, and hamster, the death occurred because of a prolonged tonic period, so no tonic period and postictal depression. In those dead animals, only the latency and clonic periods were taken into consideration.

Stastical Analysis :

The values are presented as mean ± standard deviation (SD) for latency period and seizure period for rats ,mice,hamsters. Statistical analysis was performed using one-way ANOVA, followed by postical testing of various animals , with $P < 0.05$ considered statistically significant.

Table 3 presents a time-series analysis of seizure behavior in eight individual zebrafish over a 10-minute observation period due to the slowest swimming behaviour, with data recorded at 30-second intervals

Table 3 presents the seizure behavior of zebrafish for 10 minutes, recorded every 30 seconds using a scoring system ranging from 0 (normal swimming) to 7 (death). Initially, most fish exhibit normal or mildly increased activity (scores 0–1). Within the first 2 minutes, seizure severity escalates rapidly across all animals, with most reaching clonic or tonic seizure-like behaviors (scores 4–5). This heightened seizure activity persists for several minutes. Around 6 minutes into the observation, seizure intensity begins to decline, and by the 8-minute mark, most zebrafish return to a normal or postictal state (scores 0 or 6). No fish reached the death stage (score 7), indicating recovery or stabilization by the end of the monitoring period.No zebra fish is euthanized because there is no mortality during the study .

DISCUSSION

Epilepsy is a common neurological condition characterized by abnormal electrical activity within neuronal networks of the brain. Seizures associated with epilepsy often originate in the cortical or subcortical grey matter. One contributing factor to seizure development is disruption of the blood–brain barrier, which may result from inflammatory processes affecting regions connected to the central nervous system (Sumadewi *et al.*, 2023; Rana *et al.*, 2018).

Seizures are broadly classified into two categories: those involving both cerebral hemispheres and those confined to a single hemisphere (Yin *et al.*, 2013). Glutamate plays a central role as the primary excitatory neurotransmitter in the brain. Glutamatergic neurons are widely distributed throughout the central nervous system and form connections among key regions such as the hippocampus, prefrontal cortex, and thalamus, which are also implicated in neurological disorders like schizophrenia (Gireud *et al.*, 2014; Aroniadou-Anderjaska *et al.*, 2008). Excessive glutamatergic activity contributes to neuronal hyperexcitability, and an overabundance of glutamate can trigger the onset of seizures by overstimulating neural circuits. In contrast, gamma-aminobutyric acid (GABA) functions as the principal inhibitory neurotransmitter. Its effects are mediated through two main receptor types: GABAA, which are ligand-gated ion channels, and GABAB, which are G-protein-coupled receptors. Activation of these receptors reduces neuronal excitability by increasing potassium efflux and limiting calcium influx. However, alterations in GABAergic signaling may paradoxically facilitate epileptogenesis by promoting synchronized neuronal firing and enhancing excitatory postsynaptic potentials (Yin *et al.*, 2013).

Pentylenetetrazol (PTZ) is widely used as an experimental agent to induce seizures and model chronic epilepsy. This model reproduces several features of human epilepsy, including progression in seizure severity, the number of exposures required to establish persistent seizures, and long-term stability (Ngoupaye *et al.,* 2022). PTZ acts as a chemoconvulsant by antagonizing GABAA receptors, thereby inducing seizures in various animal models such as mice, rats, hamsters, and zebrafish. It is also known to produce kindling effects, particularly in the amygdala (Dhir, 2012). Early studies demonstrated that PTZ-induced kindling is associated with reduced activity of GABA-linked chloride channels, as evidenced by decreased binding and ion uptake in cortical membrane preparations (Corda *et al.,* 1990).

Seizure activity induced by PTZ is closely linked to oxidative stress and inflammatory responses in brain regions such as the hippocampus, parietal cortex, and amygdala. In certain models, such as hypertriglyceridemic hamsters, elevated triglyceride levels appear to confer some neuroprotective effects by modulating neuronal excitability. Additionally, PTZ exposure has been shown to increase expression of immediate early genes such as c-fos in both zebrafish and rodent models (Ravikumar *et al.*, 2025; Shen *et al.,* 2024; Tavakoli *et al.*, 2023). The PTZ seizure test is commonly employed to evaluate compounds that may elevate seizure threshold under both acute and chronic conditions (Mandhane *et al.,* 2007; Dhir, 2012). Typically, seizure progression following administration is monitored over a defined period. Initial signs, including facial and forelimb clonus, appear shortly after a brief latency phase. These are followed by generalized tonic–clonic seizures, which may ultimately result in mortality if severe (Koutroumanidou *et al.,* 2013). Prolonged seizure episodes, particularly those exceeding 30 minutes, are associated with neuronal damage and apoptosis in both clinical and experimental settings (Homayoun *et al.*, 2020). At the molecular level, PTZ interferes with inhibitory neurotransmission by binding to sites on the GABAA receptor complex, thereby blocking chloride ion influx and preventing neuronal hyperpolarization. This disruption leads to sustained neuronal excitation and convulsive activity. Furthermore, PTZ exposure has been associated with reductions in both GABA levels and receptor density within the brain (Viswanatha *et al.*, 2020).

In recent years, zebrafish have emerged as a valuable model in genetic studies due to their rapid development, high reproductive rate, and cost-effectiveness. They are widely used to study neurological disorders, including epilepsy, Parkinson's disease, and Alzheimer's disease (Saleem *et al.*, 2021; Ren *et al.*, 2022). Behavioral assays in zebrafish, along with gene expression analyses such as c-Fos, are commonly applied for seizure assessment (Ravikumar *et al.*, 2025). Electrophysiological studies have demonstrated differences in neuronal activity between experimental groups. For example, hypertriglyceridemic hamsters exhibit lower frequencies of excitatory postsynaptic potentials and action potentials compared to wild-type animals under varying stimulation conditions (Ravikumar *et al.*, 2025). Additionally, variability in seizure severity has been observed in certain animal populations, such as GASH/Sal models, suggesting the presence of distinct subgroups.

Seizures may also be experimentally induced using electrical stimulation methods, such as maximal electroshock seizures (MES), or through chemical agents including β-sitosterol and evolvine. MES models are particularly useful for producing generalized tonic–clonic seizures through repeated electrical stimulation of specific brain regions (Tanna *et al.*, 2012; Wang *et al.,* 2022). Dosage and administration routes of PTZ vary across studies. In mice, intraperitoneal doses around 120 mg/kg body weight are commonly used, although lower doses such as 85 mg/kg (subcutaneous) or 100 mg/kg have also been reported (Aleshin *et al.,* 2023). Latency to seizure onset differs depending on dose and species; for example, intraperitoneal administration in mice may produce seizures in under one minute, whereas slightly longer latencies have been reported in other studies (Rahmati *et al*., 2020; Kargar Jahromi *et al.*, 2023). In zebrafish models, PTZ is typically administered by dissolving it in the surrounding water, often at concentrations around 10 mM. Seizure onset occurs rapidly, with tonic seizure activity generally appearing within 2–3 minutes and lasting approximately 60–80 seconds (Mussulini *et al*., 2013).

The findings of this study underscore the effectiveness of pentylenetetrazol (PTZ) in inducing seizures across a range of animal models, each demonstrating unique seizure profiles. The zebrafish model proved particularly useful for monitoring seizure behavior due to its transparency and rapid development, whereas rodent models offered detailed physiological and neurobehavioral data, allowing for more comprehensive analysis of seizure activity. These observations confirm the reliability and relevance of both zebrafish and rodent models for the preclinical screening of anticonvulsant agents.Importantly, the study also revealed that certain herbal extracts exhibited notable seizure-suppressing effects, suggesting their potential as alternative or complementary therapeutic options in epilepsy management.

Overall, these results support the continued use of PTZ-induced seizure models as for epilepsy research. They not only facilitate the evaluation of novel antiepileptic compounds but also provide valuable insights into the mechanisms for seizure activity, shows the way for the development of more effective and safer therapeutic interventions.

CONCLUSION

This study highlights the effectiveness of PTZ in inducing seizures across multiple animal models, each exhibiting distinct seizure characteristics. The zebrafish model proved valuable for tracking seizures, while rodents provided detailed physiological data. Results confirm the suitability of these models for

screening anticonvulsant agents. Overall, the findings support the continued use of PTZ-induced models in epilepsy research for various animal.

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DECLARATION

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Conflict of Interest : None declared

Ethical Approval : The animal experiment was carried out as per the instructions in the protocol number MB/IAEC/2024/02/02, MB/IAEC/2024/01/04, 05 given by the ethical committee of Mass Biotech, Chengalpattu, with CPCSEA registration number 2084/PO/RcBiBt/S/19/CPCSEA

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Table 1: Dose of PTZ for each species

Species	PTZ dose (mg/kg. b.wt)
Mice (Asadi-Shekaari *et al*., 2014; Shimada *et al.*, 2018)	120
Rat (Meilleur *et al*., 2003; Lotfy *et al.,* 2022)	70
Hamster	100
Zebrafish (Chitolina *et al*., 2023)	170

Table 1 lists the dosage of PTZ for different animal models like, mice, rat, hamster and, zebrafish.

Table 2: Seizure evaluation results for Mice, Rat, and Hamster

Species	Latency Period (s)	Clonic Seizure (s)	Tonic Seizures (s)	Postictal Depression (s)	Death %
Mice	58.87±14.28	50.14±70.9	23±21.7	55.83±9.34	25
Rat	53.4±11.89	24.42±14.8	18.74±14.1	42.63±28.3	37.5
Hamster	32.1±18.38	6.89±4.89	22.52±16.28	17±32.8	25

Table 3: Seizure recording of zebrafish:

	Zebra fish .No							
Time (s)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
0	0	1	0	1	1	0	0	0
30	1	1	2	4	4	4	5	4
60	2	3	4	5	5	5	5	5
90	2	4	5	4	5	5	5	5
120	2	5	5	5	5	5	5	5
150	3	5	5	4	5	5	4	5
180	4	5	5	6	5	5	4	4
210	4	5	5	6	6	5	4	6
240	3	4	5	6	6	5	4	6
270	2	6	4	6	6	5	3	6
300	2	6	4	6	6	4	0	6
330	2	6	1	6	6	4	0	6
360	0	6	2	6	6	0	0	1
390	0	1	1	0	2	0	0	0
420	0	1	1	0	2	4	0	0
450	0	0	0	0	0	2	0	0
480	0	0	0	0	0	0	0	0
510	0	0	0	0	0	0	0	0
540	0	0	0	0	0	0	0	0
570	0	0	0	0	0	0	0	0
600	0	0	0	0	0	0	0	0

Fig.1. Histopathological images of the Brain of PTZ-induced mice by using Hematoxylin and Eosin staining

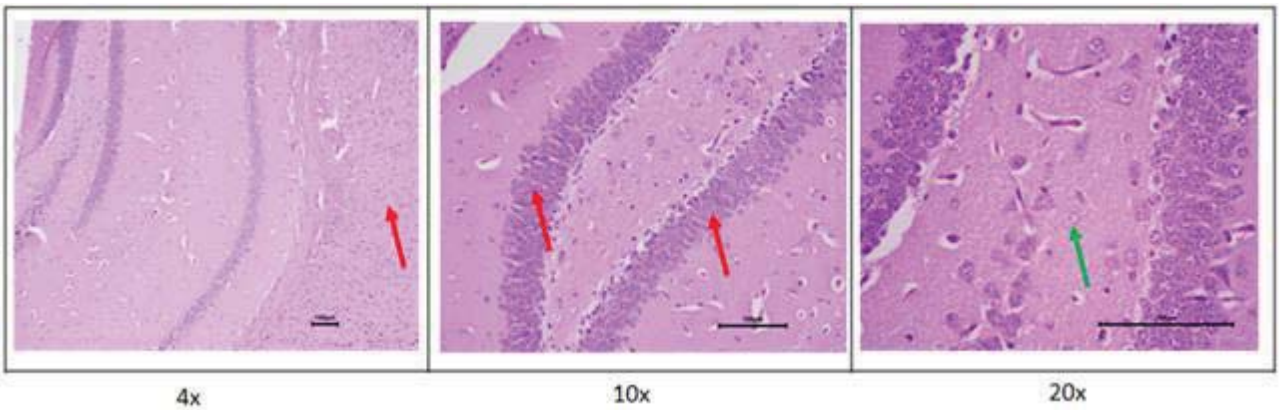

Fig.1 shows the histopathology slides of the PTZ-induced mice brain. Normal morphology of the hippocampal neuron was observed in the Brain [Red arrow] and normal morphology of the cerebral cortex region [Green arrow]