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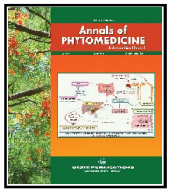

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Exploring the anticonvulsant properties of *Passiflora alata* Curtis: A comprehensive *in vitro* and *in vivo* analysis

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Abstract

Passiflora alata Curtis is increasingly recognized for its potential therapeutic applications, especially in treating CNS disorders. However, comprehensive studies evaluating its safety and convulsion efficacy are limited. This study aimed to examine the anticonvulsive activity of *P. alata* using *in vitro* and *in vivo* assays. Ellman's colourimetric method was used for the AchE inhibition assay, and pentylenetetrazole (PTZ) and maximum electroshock (MES)-induced convulsions model were used to determine the *in vivo* anticonvulsion activity.

Acute toxicity (OECD-423) testing proved no harmful effects of *P. alata*. *In vitro* AchE inhibition assay revealed that the *P. alata* leaf methanol extract (PLM) showed better inhibition. In a PTZ-induced seizure model study, PLM outperformed in anticonvulsant efficacy, evidenced by delayed seizure onset, shorter convulsion durations, and higher survival rates. PLM, at 400 mg/kg body weight dose, showed dose-dependent anticonvulsant antioxidant activity by altering SOD, CAT, and MDA levels. Partial restoration of neurotransmitter (Ach) levels, with limited impact on dopamine, was also seen. Histopathological evaluations suggest potential neuroprotective benefits. In conclusion, *P. alata*, particularly at a dose of 400 mg/kg, exhibits promising anticonvulsant properties, antioxidant activity, and neuroprotective potential, warranting further investigation into its use as an adjunct or alternative therapy in seizure management. Additional research is required to understand their therapeutic processes and long-term efficacy fully.

1. Introduction

A seizure is a sudden, transient alteration in neurological function that results from excessive and synchronized neuronal discharge in the brain. Epilepsy is characterized by recurrent, unprovoked seizures. Neurological disorders, including epilepsy, are among the most complex, posing significant challenges (Fisher *et al*., 2005).

With more than 20 medications available, successful treatment is possible for up to 70% of newly diagnosed individuals with epilepsy. The use of these medications leads to a decrease in the electrical activity of the brain by various mechanisms, such as blocking sodium or calcium channels to prevent neuronal depolarization, enhancing the function of potassium channels, inhibiting glutamate-mediated excitation, or promoting GABA-mediated inhibition (Huff and Fountain, 2011). The effectiveness of these drugs varies depending on the underlying cause of the epilepsy. Patients with no identified cause were more likely to achieve seizure control, particularly if they had an average developmental history and neurological examination. When determining which seizure medication to initiate, neurologists should consider factors such as seizure type, age, other medical conditions, and potential side effects. In cases where a detailed seizure description is unavailable, broad-spectrum medication may be chosen. Levetiracetam has gained popularity as a first-line therapy owing to its efficacy, ease of titration, and well-known side-effect profile. Previously, carbamazepine was preferred for focal seizures, while valproic acid was commonly used for generalized seizures (Mc Namara, 1999).

The recognition of herbal remedies as potential complementary or alternative medicines has become increasingly widespread (Hanish, 2022). Investigations into medicinal plants have progressed globally, demonstrating their pharmacological efficacy in various animal models of neurological conditions (Onasanwo *et al*., 2010; Nupur *et al*., 2021). Consequently, numerous herbal remedies have been the focus of extensive animal research to evaluate their potential for psychotherapy (Dandu *et al*., 2022; Ayman *et al*., 2023). These studies contribute significantly to developing innovative pharmacotherapies based on therapeutic plants and isolated active phytochemicals (Evans *et al*., 2005; Sugimoto *et al*, 2010; Galdino *et al*, 2009).

P. alata is a native Brazilian evergreen plant that serves as an essential vine with various medicinal uses (Freire *et al*., 2017; Jyoti *et al*., 2023). It is widely recognized for its hypnotic and sedative effects and has been utilized by traditional healers to treat digestive and nerve-related issues (Rotta *et al*., 2017). Phytochemicals present in *P. alata* include quadranguloside, oleanolic acid-3-sophoroside, β-carboline alkaloids, such as harmane and harmine, and steroids, such as β-sitosterol (Viera *et al*., 2022). The plant possesses pharmacological properties demonstrating immunomo-dulatory,

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antidiabetic, antitumor, antioxidant, and gastroprotective activities (Figueiredo *et al*., 2016; Amaral *et al*., 2020).

Despite the known medicinal uses and phytochemical composition of *P. alata*, there is a gap in the research specifically addressing its anxiolytic potential. This phytochemical and traditional richness motivated us to explore the anxiolytic potential of *P. alata*. This study aligns with the broader objective of discovering safer and more effective remedies for anxiety disorders, particularly those derived from natural sources.

2. Materials and Methods

2.1 Plant material

Whole herb of *Passiflora alata* Curtis was collected from Hyderabad and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati and the Voucher Specimen was (0779) preserved in the herbarium.

2.2 Extraction

1 kg of the fresh leaves, stems, and roots was shade-dried at 25-35^{o}C for seven days. The dried plant parts were powdered in a grinder. The dried plant powder was subjected to Soxhlet extraction using n-hexane, ethyl acetate and methanol to get the respective extracts. Then, maceration was carried out for the marc with water for 24 h, continuously stirring at room temperature. Then, each extract was filtered using cotton plugs, followed by Whatman No. 1 filter paper. The filtrates were then concentrated, dried under reduced pressure in the rotary evaporator, and lyophilized to get into powder form (Sujatha and Meraj Fatima, 2023). The percentage of yield was calculated using the following formula:

$$\text{Yield (g/100 g)} = (W_1 \times 100)/W_2$$

where,

W_1 = weight of the crude extract residue obtained after solvent removal

W_2 = weight of plant powder packed in the extractor

2.3 Phytochemical screening

The preliminary phytochemical screening of all the extracts of *P. alata* was performed according to the standard procedures (Harborne, 1984).

2.4 Acute toxicity studies

The acute oral toxicity study aimed to determine the therapeutic index, which was conducted according to OECD Revised Draft Guidelines 423 by the CPCSEA, India. Female wistar rats were fasted overnight and administered test extracts orally at 5, 50, 300, and 2000 mg/kg doses. The rats were observed for 4 h for behavioural, autonomic, and neurological symptoms and mortality and body weights were recorded. Behavioural changes, toxic symptoms, and mortality were monitored weekly, with body weights documented on the 7th and 14th days. As no lethality was observed, 1/5th and 1/10th of the highest doses were selected as therapeutic doses. The rats were then observed for 14 days to assess for long-term effects, and any fatalities were noted (Variya *et al*., 2019; OECD, 2002).

2.5 *In vitro* AchE inhibition assay

The acetylcholinesterase (AChE) activity was evaluated using a 96-well microplate reader based on Ellman's method (Asen *et al*., 2022). The enzyme hydrolyses the substrate acetyl thiocholine iodide (ATCI) to thiocholine and acetic acid. Thiocholine reacts with 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), forming a yellow colour that can be detected at 405 nm, with the intensity of the colour proportional to the enzyme activity. In 96-well plates, a reaction mixture containing 25 µl of 15 mM ATCI in water: 125 µl of 3 mM DTNB in buffer, and 25 µl of the plant extracts (at concentrations of 0.25, 0.5, 1, and 2 µl) was added, and the absorbance was measured at 405 nm. Following this, 25 µl of AChE solution (0.22 U/ml) was added to the wells, and the microplate was reread at the same wavelength every 1 min 10 times. Galanthamine dissolved in methanol was used as a positive control at 1 mg/ml concentration, while a blank of methanol in 50 mM Tris-HCl (pH 8) was used as a negative control. The percentage inhibition for each test solution was then calculated using the following equation:

$$\text{Inhibition (\%)} = 1 - (A_{sample}/A_{control}) \times 100$$

2.6 Pentylenetetrazole (PTZ) induced convulsion

PTZ induced seizures in rodents are believed to serve as an experimental model for myoclonic convulsions in humans. In a study involving rats, clonic convulsions were triggered by the subcutaneous administration of PTZ at a dose of 100 mg/kg. After drug administration, the rats were placed separately in a transparent plexiglass cage and monitored for 30 min for the occurrence of clonic seizures. Clonic seizures were identified as whole-body clonus lasting over 3 sec, accompanied by a loss of righting reflex (Behzadnia *et al*., 2022; Herrera *et al*., 2017).

2.7 Maximum electro shock (MES) induced convulsions

Electroshock was administered to each rat *via* ear-clip electrodes with saline or the respective drug administered at the appropriate time. The stimulus duration was 0.2 sec, and the current frequency was 50 Hz. The animals were observed for 30 min to determine whether they displayed hind limb tonic extensor (HLTE) or hind limb tonic flexion (HLTF). The endpoint of this model was defined as the protection against seizures. Animals that regain their normal exploratory behaviour within 10 sec of stimulation are protected (Mombeini *et al*., 2020; Loshali *et al*., 2021).

2.7.1 Estimation of biochemical parameters in rat brain homogenate

Determination of SOD, CAT, and MDA levels in the brain homogenate was performed according to the standard protocols available with the kits (Pasha and Sadasivadu, 1984; Marklund and Markund, 1974; Aebi, 1983).

2.7.2 Estimation of neurotransmitters

Ach levels in rat brain homogenate were estimated by the Hestrin and Stepankova method, and Dopamine levels by the method of Guo spectrophotometric method (Štìpánková *et al*., 2005; Guo *et al*., 2009).

2.8 Statical analysis

The results of the experiments and observations were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed with GraphPad Prism version 9.0, using relevant tools to understand significance.

3. Results

3.1 Acute toxicity studies

Acute toxicity studies revealed the safety of *P. alata*, showing no toxicity symptoms at 2,000 mg/kg body weight for 14 days of observation. The 1/5th dose, *i.e.*, 400 mg/kg body weight, is selected as the therapeutic dose for future *in vivo* studies (Table 1).

Table 1: Acute toxicity response on body weight

Sample	Body weights									Signs of toxicity	No. of deaths
	PSM			PLM			PLE				
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14		
Control	218.5±2.71	219.8±2.5	230.1±2.91	219.5±2.71	229.4±2.56	230.1±1.91	228.5±1.71	239.8±2.56	240.1±1.91	None	0
5 mg/kg	219±2.74	217.8±2.89	238.6±2.43	212.8±2.43	223.5±2.60	236.5±2.01	223.6±1.1	233.1±2.70	246±2.84	None	0
50 mg/kg	220.8±2.09	222.3±11.47	239.5±2.56	229±2.89	236.12±3.70	235.66±3.7	227.8±1.0	235±2.30	247.3±2.71	None	0
300 mg/kg	215.1±1.31	223±11.7	241±2.6	211.5±3.05	229.6±3.16	240.66±3.93	222±1.44	239.8±2.98	259.3±2.73	None	0
2000 mg/kg	215.6±1.03	227.3±2.51	247.6±2.31	218±2.71	227.8±2.95	249±2.44	229.8±2.75	238.5±2.59	251.8±2.54	None	0

3.2 *In vitro* AchE inhibition assay

Ellman's colourimetric method is widely employed to evaluate the anticholinesterase potential of compounds, including plant and natural source extracts. This study assesses the anticholinesterase potential of *P. alata* using a 96-well microplate. Acetylcholine (Ach) plays a critical role in the pathophysiology of epilepsy and has a significant effect on the central nervous system. Elevated Ach levels can increase cortical sensitivity to external stimuli, resulting in decreased corticocortical communication and increased attention. However, excessive Ach signalling can also cause symptoms related to anxiety and depression.

By evaluating anticholinesterase potential, this study aimed to determine whether these extracts could modify Ach levels in the central nervous system, affecting epilepsy and related disorder symptoms. Galantamine, leaf ethyl acetate extract (PLE), leaf methanol extract (PLM), and stem methanol extracts were evaluated at concentrations ranging from 0.25 µg/ml.

When comparing the IC_{50} values, it can be seen that PLE had an IC_{50} value of 3.44 µg/ml, PSM had IC_{50} value of 4.12 µg/ml and PLM had IC_{50} value of 3.00 µg/ml, suggesting that PSM is the second-most potent inhibitor of acetylcholinesterase among the four compounds, followed by PLE, and finally PLM, which showed the slightest inhibition of the enzyme. Regarding the dose-response relationship, both galantamine and PSM showed a similar trend of increased inhibition with increasing doses. Galantamine showed 89.0% inhibition at the highest dose tested, while PSM showed 58.33% inhibition (Table 2 and Figure 1).

Table 2: AchE inhibition assay

Concentration	PLE	PLM	PSM	Galantamine
0.25	11.67 ± 2.52	13 ± 1.0	17.67 ± 2.08	22.33 ± 3.00
0.5	18.33 ± 1.53	15.67 ± 1.00	23.33 ± 2.52	29.33 ± 2.65
1	26.67 ± 1.53	19.33 ± 1.53	33.00 ± 2.08	46.33 ± 1.53
2	42.00 ± 1.15	46.0 ± 2.64	26.3 ± 2.53	68.67 ± 1.53
5	63.33 ± 2.08	67.33 ± 2.08	58.33 ± 3.51	89.0 ± 2.00
Y=mx+c	y = 10.394x + 14.21	y = 9.993x + 19.977	y = 9.971x +8.149	y = 3.2x+28.967
IC_{50} values	3.44	3.0	4.12	1.59

All values are expressed as mean ± SEM.

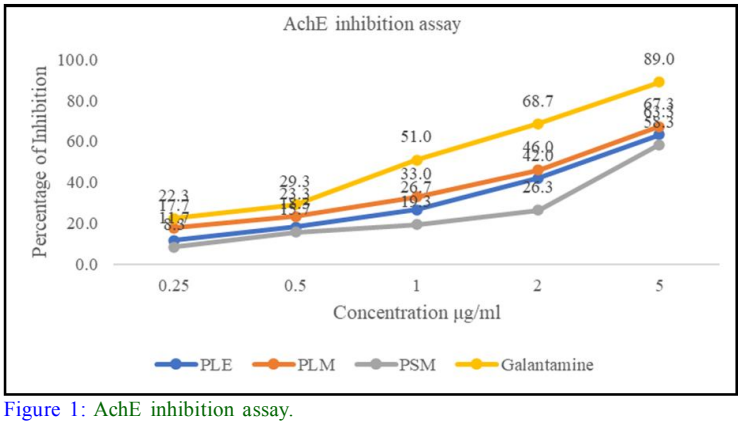

Figure 1: AchE inhibition assay.

3.3 Maximal electro shock (MES) induced convulsions model

The experimental results demonstrate the impact of the methanolic leaf extract of *P. alata* (PLM) on MES (maximal electroconvulsive shock) induced convulsions in rats. The study comprised three groups of rats: Group II received standard diazepam as a positive control, Group III received PLM at 200 mg/kg, and Group IV received PLM at 400 mg/kg. The 400 mg/kg dose of PLM significantly reduced hind limb tonic extensor (HLTE) and hind limb tonic flexion (HLTF) duration. The results indicate that 400 mg/kg PLM was the most effective in reducing the duration of HLTE and HLTF. However, there was no effect on the clonus (Table 3a-3d and Figure 2).

Table 3a: Effect of *P. alata* on the duration of HLTE in MES induced convulsion

Treatment	Duration of HLTE	
	Before treatment	After treatment
Group I: Normal control	14.7 ± 0.89	13.9 ± 0.84^{ns}
Group II: Standard diazepam (1mg/kg per oral)	14.4 ± 0.87	0.0
Group III: (PLM-200 mg/kg, per oral)	14.1 ± 0.85	8.4 ± 0.24**
Group IV: (PLM-400 mg/kg, per oral)	14.7 ± 0.89	4.0 ± 0.32***

n=5, All values are expressed as mean ± SEM. The grouped paired *'t'* test was used to compare before and after the treatment. ***p<0.001, **p<0.01, significance difference before and after the treatment, ^{ns} non-significance difference before and after the treatment.

Table 3b: Effect of *P. alata* on the duration of HLTF in MES induced convulsion

Treatment	Duration of HLTF	
	Before treatment	After treatment
Group I: Normal control	6.47 ± 0.4	6.14 ± 0.38 ^{ns}
Group II: Standard diazepam (1mg/kg per oral)	6.4 ± 0.4	0.0
Group III: (PLM-200 mg/kg, per oral)	6.54 ± 0.4	4.4 ± 0.5*
Group IV: (PLM-400 mg/kg, per oral)	6.34 ± 0.39	2 ± 0.32***

n=5, All values are expressed as mean ± SEM. The grouped paired *t'* test was used to compare before and after the treatment. ***p<0.001, *p<0.05 significance difference before and after the treatment, ^{ns} non-significance difference before and after the treatment.

Table 3c: Effect of *P. alata* on the Clonus in MES induced convulsion

Treatment	Clonus	
	Before treatment	After treatment
Group I: Normal control	13.53 ± 1.13	12.85 ± 1.07 ^{ns}
Group II: Standard diazepam (1mg/kg per oral)	13.4 ± 1.12	17.2 ± 0.66 ^{ns}
Group III: (PLM-200 mg/kg, per oral)	13.53 ± 1.13	16.6 ± 0.4 ^{ns}
Group IV: (PLM-400 mg/kg, per oral)	13.27 ± 1.11	15.4 ± 0.24 ^{ns}

n=5, All values are expressed as mean ± SEM. The grouped paired *'t'* test was used to compare before and after the treatment. ^{ns} non-significance difference before and after the treatment.

Table 3d: Effect of P. alata on the Stupor in MES induced convulsion^{s}

Treatment	Stupor	
	Before treatment	After treatment
Group I: Normal control	135.36 ± 1.59	137.4 ± 1.61 ^{ns}
Group II: Standard diazepam (1mg/kg per oral)	131.2 ± 2.29	57.4 ± 1.43***
Group III: (PLM-200 mg/kg, per oral)	136.4 ± 2.38	100.2 ± 2.89***
Group IV: (PLM-400 mg/kg, per oral)	133.8 ± 2.33	80.0 ± 0.89***

n=5, All values are expressed as mean ± SEM. The grouped paired *'t'* test was used to compare before and after the treatment. $^{***}p$<0.01 $^{**}p$<0.05 $^{*}p$<0.5 significance difference before and after the treatment, ^{ns} non-significance difference before and after the treatment.

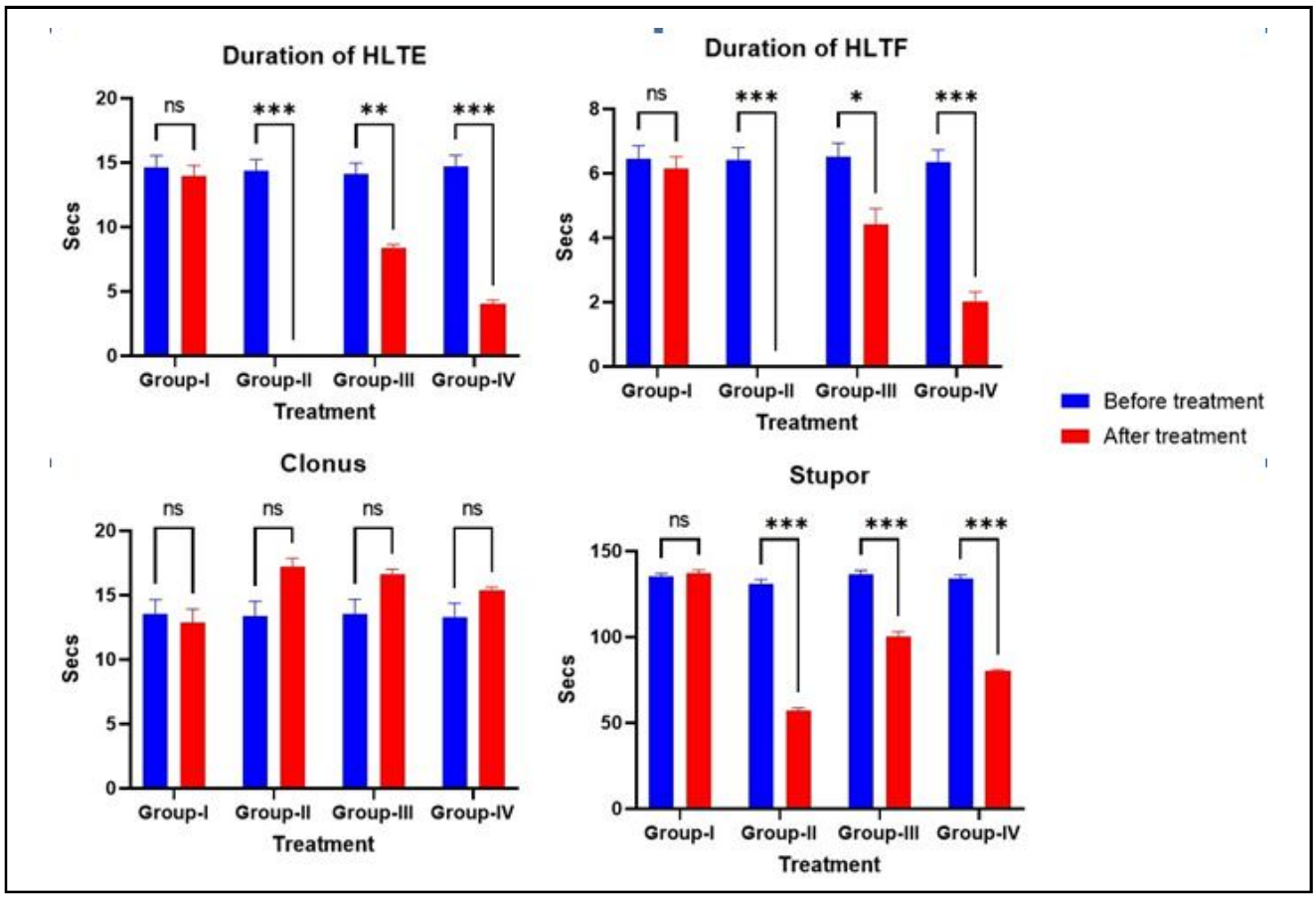

Figure 2: Effect of methanolic leaf extract of *P. alata* in MES induced convulsion.

3.4 Pentylenetetrazole (PTZ) induced seizures model

The experimental animals displayed convulsions and clonic and tonic seizures after PTZ administration. When comparing the results of Group III (standard diazepam) with Group IV (PLM-200 mg/kg) and Group V (PLM-400 mg/kg), it can be seen that standard diazepam demonstrated better anticonvulsant effects, followed by both groups receiving PLM. The onset of seizures in Group III was significantly delayed (310 ± 0.73 sec) compared to Group IV (235 ± 1.46 sec) and Group V (271 ± 1.2 sec). The duration of jerks in Group III was also shorter (424 ± 1.61 sec) compared to Group IV (584 ± 0.92 sec) and Group V (524 ± 1.43 sec). All the animals in Group III survived, while only 66.67% and 83.3% of the animals in Group IV and Group V, respectively, survived (Table 4 and Figure 3).

Group V, which received PLM at a dose of 400 mg/kg per oral, showed the best results compared to Group III (standard diazepam) regarding the anticonvulsant activity. The onset of seizures in Group V was delayed compared to the disease control group (Group II) and reduced compared to the group treated with 200 mg/kg orally (Group IV). The duration of jerks in Group V was also decreased compared to the disease control group and was shorter than in Group IV. Additionally, the percentage of animals that survived in Group V was 83.3%, higher than the disease control group (0%) and higher than the group treated with 200 mg/kg per oral (80%). These results highlight the potential of 400 mg/kg per oral as an effective anticonvulsant drug.

Table 4: Effect of methanolic leaf extract of *P. alata* in PTZ-induced convulsion.

Treatment	Onset of seizures (Sec)	Duration of jerks (Sec)	Animals survived	Percentage of protection
Group I: Normal group	323.4 ± 1.03	399 ± 1.88	5/5	-
Group II: Disease Control	195 ± 1.58	722 ± 2.06	0/5	0
Group III: Standard diazepam (1mg/kg per oral)	310 ± 0.73*	424 ± 1.61*	5/5	100
Group IV: (PLM-200 mg/kg, per oral)	235 ± 1.46*	584 ± 0.92*	4/5	80
Group V: (PLM-400 mg/kg, per oral)	271 ± 1.2*	524 ± 1.43*	4/5	80

n=5, All values are expressed as mean ± SEM, statistical analysis by One-way ANOVA followed by Dunnett's test, *p<0.05 indicates statistical significance, comparison with disease control

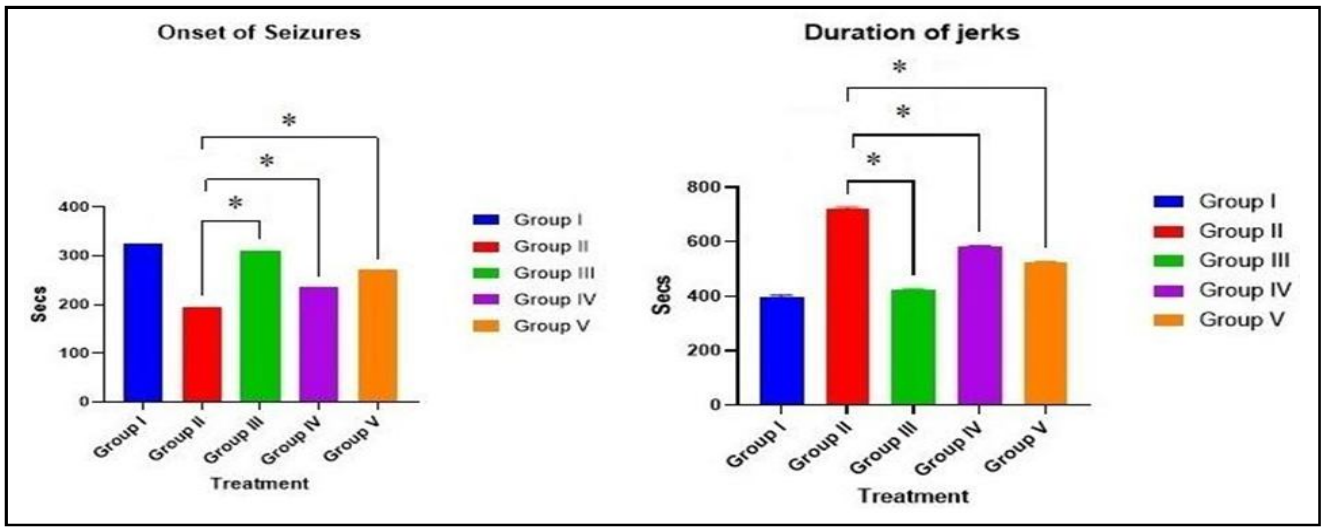

Figure 3: Effect of methanolic leaf extract of *P. alata* in PTZ induced convulsion

3.4.1. Effect on biochemical parameters

The results of the experiments show the effect of different treatments (PTZ, Standard Diazepam, PLM-200 mg/kg, and PLM-400 mg/kg) on the levels of SOD, CAT, and MDA in the tissue. High levels of SOD and CAT indicate increased antioxidant activity, while high levels of MDA indicate oxidative stress.

The PLM-200 mg/kg (26.4 ± 2.07 and 50.6 ± 1.14) and PLM-400 mg/kg (39.4 ± 2.40 and 113 ± 2.16) groups showed intermediate levels of SOD and CAT, suggesting intermediate antioxidant activity. PLM 400 mg/kg also showed decreased levels of MDA (2.1 ± 0.05), indicating that PLM-400 mg/kg had an effect on reducing oxidative stress, next to standard diazepam treatment (Table 5 and Figure 4).

Table 5: Effect on biochemical parameters

Treatment	SOD (U/mg tissue)	CAT (U/mg tissue)	MDA (nmol/gm tissue)
Group I: Normal group	59.2 ± 2.16	193 ± 3.80	1.36 ± 0.11
Group II: PTZ group (100 mg/kg)	16.8 ± 3.27$^{###}$	34.8 ± 3.11$^{###}$	3.84 ± 0.21$^{###}$
Group III: Standard diazepam (1mg/kg per oral)	39.4 ± 2.40***	136 ± 1.34***	1.8 ± 0.23***
Group IV: (PLM-200 mg/kg, per oral)	26.4 ± 2.07**	50.6 ± 1.14**	2.5 ± 0.19**
Group V: (PLM-400 mg/kg, per oral)	46.2 ± 1.30***	113 ± 2.16***	2.1 ± 0.05**

n=5, All values are expressed as mean ± SEM, statistical analysis by One way ANOVA followed by Dunnett's multiple comparison test, $^{***}p<0.001$, $^{**}p<0.01$ when compared to the PTZ group

$^{###}P<0.001$ when compared to the Normal group

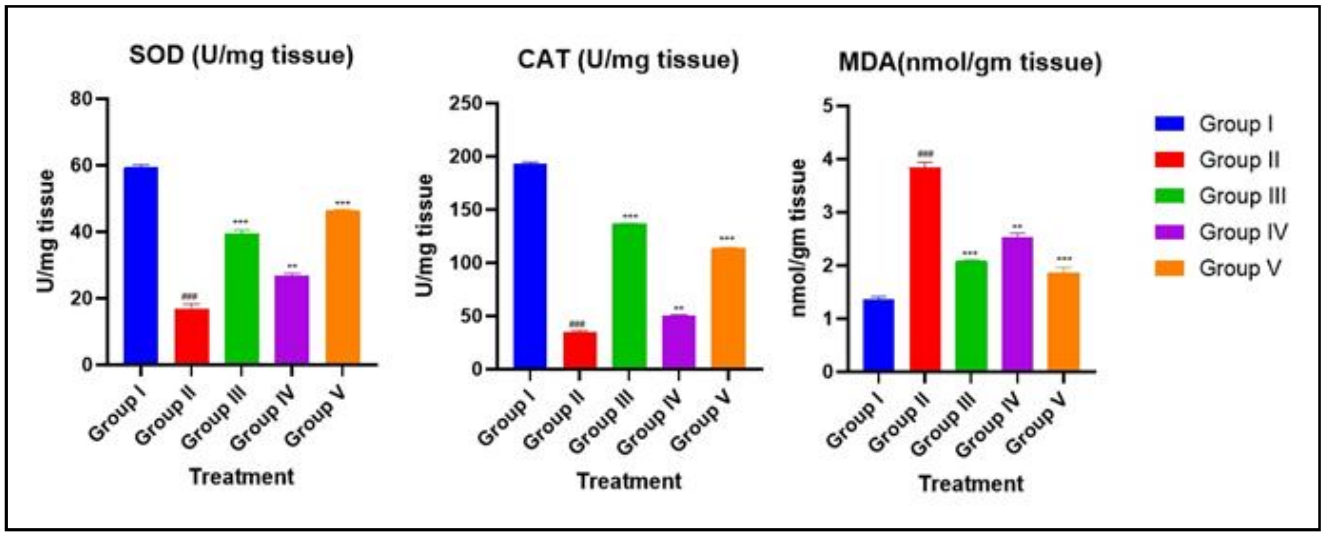

Figure 4: Effect on biochemical parameters.

3.4.2 Estimation of neurotransmitters

The normal group showed baseline levels of 9 µg/ml. In the PTZ group, which models seizure conditions, there was a notable decrease in Ach levels to 3.8 µg/ml. Diazepam, partially restored Ach levels to 7.6 µg/ml. PLM, at a dose of 200 mg/kg, slightly increased Ach levels to 4.2 µg/ml, and at a higher dose of 400 mg/kg, further increased them to 6.2 µg/ml. For dopamine levels, the normal group had 0.14 µg/ml. The PTZ group showed increased dopamine levels at 0.32 µg/ml. Diazepam treatment brought the dopamine levels down to 0.19 ìg/ml. With PLM treatment, at the higher dose of 400 mg/kg, the levels were decreased to 0.27 µg/ml. This pattern suggests that PLM does not significantly affect the brain dopamine levels (Table 6 and Figure 5).

Table 6: Effect on neurotransmitter levels

Treatment	Ach levels (µg/ml)	Dopamine levels (µg/ml)
Group I: Normal group	9 ± 0.71	0.14 ± 0.01
Group II: PTZ group (100 mg/kg)	3.8 ± 0.37$^{###}$	0.32 ± 0.04 $^{###}$
Group III: Standard diazepam (1mg/kg per oral)	7.6 ± 0.51 ***	0.19 ± 0.01 ***
Group IV: (PLM-200 mg/kg per oral)	4.2 ± 0.37 ^{ns}	0.3 ± 0.01^{ns}
Group V: (PLM-400 mg/kg, per oral)	6.2 ± 0.37**	0.27 ± 0.02 ^{ns}

n=5, All values are expressed as mean ± SEM, statistical analysis by One way ANOVA followed by Dunnett's multiple comparison test, ***p<0.001, **p<0.01^{ns} non-significant, whereas, $^{###}p$<0.001 is compared to the normal group.

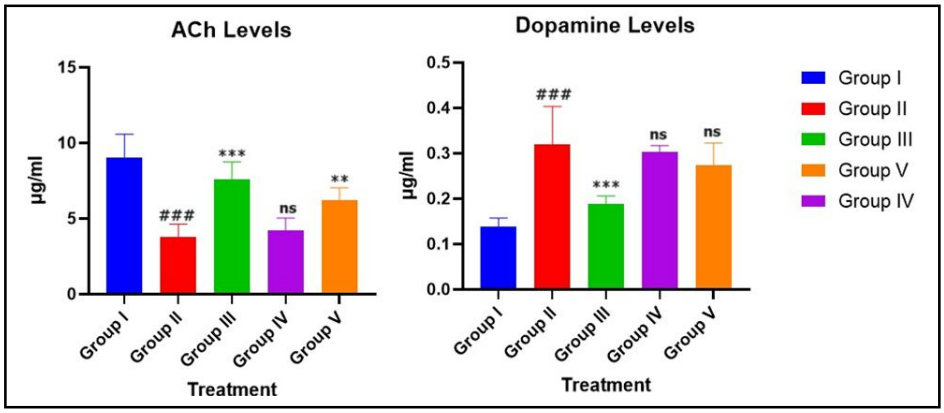

Figure 5: Effect on neurotransmitter levels

3.4.3. Histopathology of brain

The study investigated the effects of convulsion inducing chemicals on the brain histology of experimental animals and evaluated the recovery of brain function after treatment with diazepam and *P. alata* (PLM).

Group I: Normal control: The hippocampal region of the brain shows normal architecture with no neurodegeneration and necrotic lesions. The karyopyknotic characters with condensed and denatured chromatids were not observed (Figure 5a).

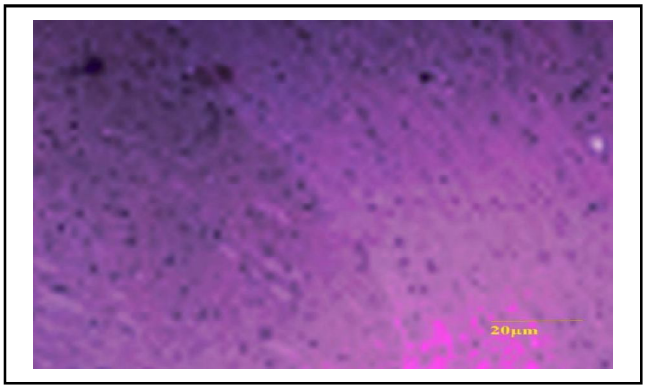

Figure 5a: Regular histology of rat brain.

Group II: PTZ group (100 mg/kg): The hippocampal region of the brain showing abnormal architecture with neurodegeneration and necrotic lesions. The karyopyknotic characters with condensed and denatured chromatids were also observed. Pyramidal cells are clustered in a dense layer with minimal neuropil integration (Figure 5b).

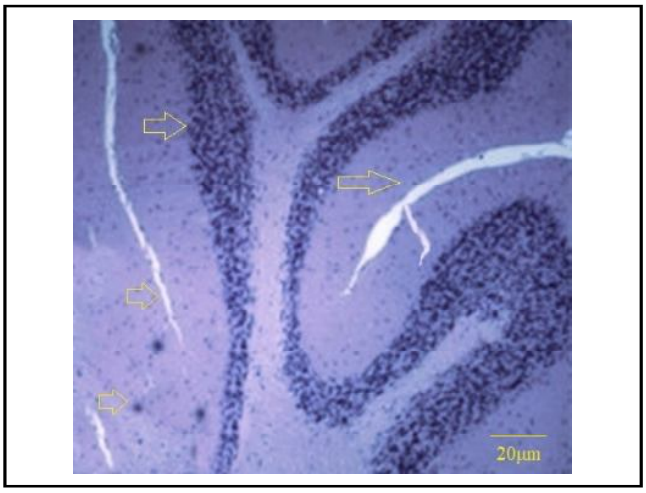

Figure 5b: Effect of PTZ on histology of rat brain.

Group III: Standard diazepam (1mg/kg per oral): Pyramidal cells from the same anatomical regions showed karyolitic pale-coloured nuclei compared to the negative control group. The dentate gyrus's granule cell layer was thinner, and the basophilic cytoplasm and attenuated pyknotic nuclei were highly stained. Some granule cells had degenerated, some had karyolitic nuclei, and have a stunning halo appearance (Figure 5c).

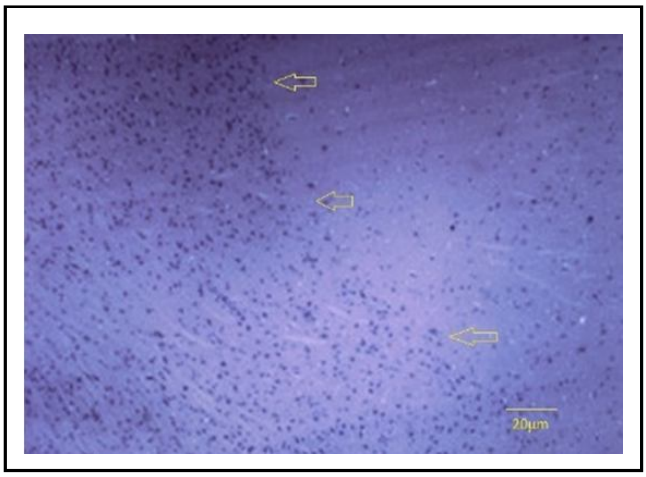

Figure 5c: Effect of diazepam on histology of rat brain.

Group IV: (PLM-200 mg/kg**,** per oral**):** Pyramidal cells with attenuation and dark staining were reduced. The granule cell layer exhibited hypertrophy in contrast to the positive controls. Only a few small, strongly stained, pyknotic nuclei remained in the granule cells. Both pyramidal and granule cells showed an increase in Nissl staining compared to diseased animals. There was a reduction in astrocytes as compared to the two control groups. In those astrocytes identified, shorter and smaller cytoplasmic processes were seen (Figure 5d).

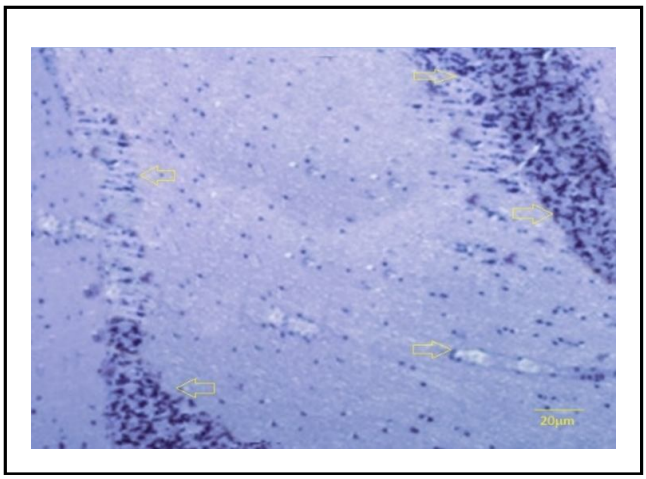

Figure 5d: Effect of PLM-200 mg/kg on histology of rat brain.

Group V: (PLM-400 mg/kg**,** per oral**):** It was possible to see almost regular microscopic characteristics. Pyramidal cells from these areas had central vesicular nuclei and basophilic cytoplasm. Glial cells and intensely stained interneurons were found at the molecular and polymorphism levels. There was a decrease in the number of astrocytes and an increase in Nissl granule density (Figure 5e).

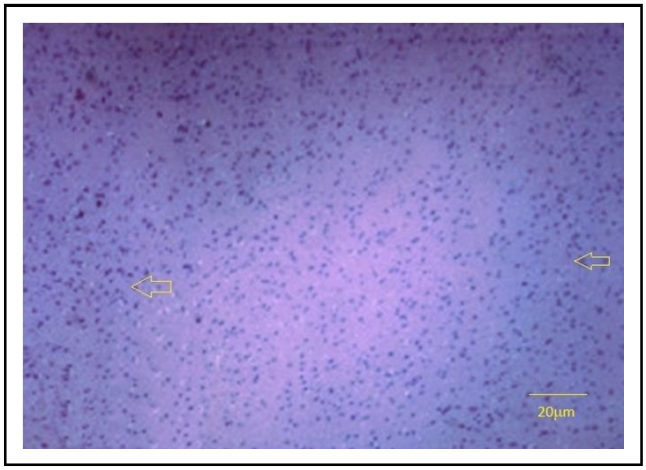

Figure 5e: Effect of PLM-400 mg/kg on histology of rat brain.

4. Discussion

The acute toxicity studies of *P. alata* have been critical in establishing its safety profile. Our study demonstrated no toxicity symptoms at a 2,000 mg/kg body weight dosage, suggesting its safety for consumption. These results are consistent with previous studies, such as Jane *et al*. (2010), who reported safety of up to 4800 mg/kg body weight in wistar rats. Our results confirmed the safety of *P. alata* leaves.

The anticonvulsant activity of *P. alata* was assessed using *in vitro* AChE inhibition experiments and *in vivo* anticonvulsant activity models. The data revealed a convergence of findings highlighting the therapeutic potential of *P. alata* extracts, especially the methanolic leaf extract (PLM), for treating epilepsy and related illnesses. This study investigated the *in vitro* AChE inhibition assay and used Ellman's colourimetric method to examine the anticholinesterase capability of *P. alata* extracts. Acetylcholine (Ach), which affects cortical sensitivity and signalling, plays a crucial role in the pathogenesis of epilepsy. Hence, determining the anticholinesterase activity of *P. alata* extracts is essential for understanding their therapeutic potential. These findings demonstrate that the extracts have unique efficacy, with PLM exhibiting considerable inhibitory effects, suggesting that Ach levels may be modulated. Lima *et al*. (2018) reported the AChE inhibition potential of *P. edulis* fruit, and our results align with their experimental findings.

When comparing the *in vitro* and *in vivo* anticonvulsant activity results, the therapeutic potential of *P. alata* became clearer. Researchers have used *in vivo* models, such as PTZ-induced seizures and MES-induced convulsions, to gain valuable insights. These models highlight how PLM at a 400 mg/kg dose effectively reduces convulsive activity. In the PTZ model, PLM delayed the onset of seizures, decreased the jerk duration, and demonstrated a greater survival rate than the other groups. In the MES model, the duration of HLTE and HLTF decreased considerably.

Based on these findings, *P. alata*, particularly its methanolic leaf extract (PLM), is a promising candidate for anticonvulsant therapy. The reduced convulsive activity and higher survival rates in the *in vivo* investigations were consistent with AChE inhibition, as demonstrated by the *in vitro* data. This data suggests a potential shift in Ach levels, which is essential for treating epilepsy and related symptoms.

The combination of *in vitro* and *in vivo* experiments underscores the potent anticonvulsant potential of *P. alata*, paving the way for future scientific exploration and treatment advancements in epilepsy. The alignment of anticholinesterase potential and *in vivo* anticonvulsant efficacy indicates how *P. alata* regulates neurological processes as a starting point for innovative treatment approaches.

All extracts of *P. alata* displayed anticonvulsant activity in the *in vitro* AChE inhibition assay and PLM showed significant reductions in HLTE and HLTF durations in various models, such as the MES-induced convulsions model and PTZ-induced seizure model. PLM, administered orally at 400 mg/kg, showed superior anticonvulsant activity compared to the standard diazepam.

As the primary antioxidant enzymes in the brain, catalase (CAT) and superoxide dismutase (SOD) are essential for the treatment of oxidative stress and related disorders. MDA is an indicator produced by the lipid peroxidation in oxidative stress. The PTZ has increased the MDA and decreased SOD and CAT levels in the experimental animals. The standard Diazepam and methanolic leaf extract of *P. alata* (PLM) has reverted the elevated MDA concentrations and revived the antioxidant enzymes (SOD and CAT). Significant enhancement in the biochemical parameters has been achieved at a concentration of 200 and 400 mg/kg body weight of PLM. Ach levels were decreased in the PTZ group, and the diazepam restored the Ach levels and Dopamine levels in the brain. The low levels of Ach in the PTZ group indicate seizures in the animals. The PLM significantly improved the Ach levels and did not affect the Dopamine levels in the brain homogenate. This indicates the protective effects of *P. alata* against seizures and helps to recover from emotional and behavioural abnormalities. The brain histology (20 µm) of the convulsion animals has shown abnormal hippocampal region architecture with neurodegeneration and necrotic lesions (A). The karyopyknotic characters were also observed in the hippocampi of disease groups. The administration of convulsion-triggering chemicals can denature the chromatid and condense it. The Diazepam-treated groups (B) have recovered from all the above abnormalities and hippocampal aberrations.

Similarly, treating *P. alata* (C and D) causes improvement in brain histology, which was dose-dependent. Higher dose treated groups (D) have recuperated from the hostile architecture of the hippocampal histology and maintained the normal physiological functions of the brain. These results show that the PLM can reverse abnormal neurological behaviour in experimental animals.

5. Conclusion

The *in vitro* assay results revealed that PLM had a lower impact on AChE than the other extracts. The anticonvulsant effect of PLM was tested using two *in vivo* models: Maximal electroshock and Pentylenetetrazole models. PLM demonstrated a decrease in the duration of HLTE and HLTF and fewer stupors. Concerning anticonvulsant activity, 400 mg/kg body weight of PLM orally showed comparable results.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article

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