Phytomedicine Plus 5 (2025) 100773

Contents lists available at ScienceDirect

Phytomedicine Plus

journal homepage: www.sciencedirect.com/journal/phytomedicine-plus

Evaluation of antiepileptic properties of herbal mix of different combinations by PTZ-induced mouse model

P.S. Venkatesan [a,*], S. Sundaresan [b], M. Eswarya [b], M. Madhavaselvi [a], R. Renuka [a]

[a] Mass Biotech Private Limited, Padi, Chennai, Tamil Nadu-600050, India
[b] SRM MCH, SRM IST, Kattankulathur, Tamil Nadu, India

ARTICLE INFO

Keywords:
Evolvulus alsinoides
Withania somnifera
Matricaria recutita
Epilepsy
PTZ
Mice

ABSTRACT

Background: Epilepsy is a neurological disorder characterized by convulsive seizures. Between 50 and 70 million people worldwide are impacted, and safer medications with superior anticonvulsant qualities and greater accessibility are still needed. Antiepileptic drug (AED) side effects continue to be a significant concern despite advances in pharmacotherapy, as they can lower quality of life and adherence. Herbal medicines are becoming more and more popular as complementary and alternative therapies as a result.

Methods: Using a mouse seizure model caused by PTZ (Pentylenetetrazole) of dose 120 mg/kg.b.wt, this study examines the anticonvulsant efficacy of a traditional medicinal plant called *Withania somnifera, Matricaria recutita and Evolvulus alsinoides* which are commonly called as Ashwagandha, Chamomile and Morning glory respectively. The herb was mixed with 1.5 % Carboxymethyl cellulose (CMC) and given to mice with various dosage levels and in combination.

Results: The study demonstrates that *Evolvulus alsinoides, Withania somnifera*, and *Matricaria recutita*, possesses significant anticonvulsant properties in a PTZ-induced seizure model in mice. Combination of all three showed highest latency of 168.25 ± 30.4s and seizure period of 3.82 ± 7.65 s

Introduction

Recent breakthroughs in genomics and neuroimaging have revealed pathways and neural network dysfunctions associated with a neurological disorder called Epilepsy. It is often categorized as a chronic illness in which many neurons fire rapidly and simultaneously leading to an excessive electrical activity monitored in the brain, often resulting in a seizure. In addition to that, the disorder is also commonly noted to have behavioral, cognitive, psychological, and social repercussions (Bastos and Cross, 2020). There are various causes and types of seizures associated with Epilepsy with which the choice of antiseizure drugs (ASD) used varies (Hakami, 2021). ASDs are effective due to their anticonvulsant properties, such as modulation of ion channels, preventing glutamate-mediated neurotransmitter interactions, and boosting inhibitory GABA transmissions. However, about one-third of epileptic patients frequently acquire an ASD resistance and others exhibit a variety of adverse effects such as pancreatitis, audio-visual problems, and even liver and kidney deteriorations, showing that ASDs have undesirable side effects along with an inefficiency in controlling or reducing epileptic seizures. The limitations of current ASD therapies highlight the need for a novel approach to treat the disorder while minimizing their side effects (Akyüz et al., 2021).

Medicinal plants have long been a source of bioactive compounds with therapeutic potential, offering a promising avenue for the development of novel epilepsy treatments. Among these, *Evolvulus alsinoides* (Morning Glory), *Matricaria recutita* (Chamomile), and *Withania somnifera* (Ashwagandha) have garnered attention for their neuroprotective and anticonvulsant properties. These plants have been extensively used in traditional medicine systems, and their selection in this study is deeply rooted in their historical applications and ethno pharmacological evidence.

A member of the Convolvulaceae family, *Evolvulus alsinoides* is a medicinal plant, widely found in the tropical and subtropical regions and is traditionally used in Ayurveda as an able agent to promote wound healing, memory and learning, neuroprotection, hepato-protection, cardio-protection, anti-diabetic activity, asthma, and epilepsy (K et al., 2023; Awere et al., 2025). It is also known as Vishukranthi in local language (Wagh and Dhuri, 2023). Its use in the management of neurological disorders, including epilepsy, is well-documented in ancient Ayuvedic texts such as the *Charaka Samhita* (K et al., 2023;

* Corresponding author at: Mass Biotech Private Limited, Padi, Chennai, 600050, India.
E-mail address: massbiotech2019@gmail.com (P.S. Venkatesan).

https://doi.org/10.1016/j.phyplu.2025.100773

Available online 7 March 2025

Mehla et al., 2020). Phytochemical studies have identified bioactive compounds such as- scopoletin, umbelliferone, and flavonoids that contribute to its therapeutic effects (Alqahtani et al., 2022; Gomathi et al., 2015, K et al., 2023; Padi et al., 2022). These compounds are believed to modulate neurotransmitter systems and reduce oxidative stress, providing a scientific basis for its traditional use in epilepsy treatment. Morning Glory plant paste with mustard oil (3:1) is applied on head for promoting growth of hair and the plant juice administered orally to treat general weakness and loss of memory. The plant contains betaine, alkaloids, hydrocarbons and β-sitosterol, evolvine, etc (Sahu and Gupta, 2014).

Matricaria recutita (Chamomile) also called as Seemai Samandhi in tamil, contains around 120 components and produces upto 2 % volatile oil. The primary components of the oil include terpenoids, primarily sesquiterpenes, and α-bisabolol. The major flavonoids present in the plant are quercetin, patuletin, luteolin, and apigenin (Ali Esmail Al-Snafi and Lawahidh Fali Hasham, 2023). Ingredients in chamomile have been found to have significant effects on the CNS (central nervous system) disorders like AD (Alzheimer's disease) and epilepsy (Sah et al., 2022). Out of them, apigenin appeared to decrease neurodegeneration at the maximum rate within the hippocampus as suggested by Immunohistological studies, It was even shown to amelorate memory shortfalls in pre-clinical trials epilepsy in an anticonvulsant study conducted by Hashemi et al. on mice models (Hashemi et al., 2019). These findings align with chamomile's traditonal use as a natural remedy for CNS disorders, supporting its selection for this study.

Similarly, *Withania somnifera*, natively known as Ashwagandha, is a multipurpose restorative plant of the family Solanaceae. Clinical and preclinical trials suggest the ability to cure hepatotoxicity, Parkinson's, hyperlipidemia, and neurological disarranges (Saleem et al., 2020). Phytochemical investigation of W. somnifera uncovered the nearness of pharmacologically dynamic steroidal lactones named withanolides, along with major phytochemicals including saponins, steroids, flavonoids, phytophenols, and glycosides. Withanine, a bunch of alkaloids disconnected from the roots of the plant, shapes 38 % of the full weight of alkaloids. It has an antioxidative component that increments the levels of gamma-aminobutyric corrosive (GABA) and cortical muscarinic acetylcholine (Ach), as well as upgrading neurite recovery all through the brain (Moghimi and Harini, 2022). This plant has been specifically recommended in Ayurvedic texts for the management of Apasmara (epilepsy) due to its ability to balance Vata and Kapha, which are believed to be implicated in the pathogenesis of the disorder (Adiga et al., 2024, Tanna et al., 2012)

As such, these plants- *Evolvulus alsinoides, Marticaria recutita*, and *Withania somnifera*- present a promising avenure in the treatment of epilepsy and related seizure disorders. Furthermore, the selection of these herbs is supported by their traditional use and extensive preclinical evidence (Adiga et al., 2024; Mehla et al., 2020). For instance, *Withania somnifera* has been revered in Ayurveda for its adaptogenic and neuroprotective properties, with clinical and preclinical studies highlighting its potential in treating neurodegenerative and seizure disorders (Moghimi and Harini, 2022; Saleem et al., 2020).

It is also noteworthy that when combined, these herbs may exhibit synergistic effects, enhancing their individual therapeutic properties. A representative ase is that the GABAergic activity of *Withania somnifera* may complement the glutamate-modulating effects of Matricaria recutita, while the antioxidative and neuroprotective properties of Evolvulus alsinoides could further mitigate seizure-induced neuronal damage (Auxtero et al., 2021).. Such synergism could potentially improve the efficacy of the herbs when used in combination, in comparison to their individual efficacy, and alo reduce the required doses of the individual compounds, thus minimizing adverse effects.

Pentylenetetrazol (PTZ) is a well-established chemoconvulusant used to induce epilepsy in rodent models. It acts by antagonizing the gamma-aminobutyric acid (GABA) receptors, leading to reduction in inhibitory neurotransmission and an increase in neuronal excitability (Bastos and Cross, 2020). This mechanism is the foundation for mimicking seizure activity, thus making it a reliable agent commonly The administration of PTZ reliably induces acute seizures in a dose-dependent manner thus forming the foundation for mimicking seizure activity (Shimada and Yamagata, 2018). This model thus provides a robust platform for studying the underlying pathophysiology of epilepsy, testing of more reliable ASDs, and explores neruoprotective interventions. PTZ-induced seizures are characterized by distinct, quantifiable behavior and electrophysiological responses, ensuring reproducibility efficiency (Van Erum et al., 2019. In this study, PTZ was administered to mice to create an acute epilepsy model, facilitating an effective evaluation of potential antiepileptic effects of the test compounds

Materials and methods

Preparation of herbal drugs

The herbal drug used are *Evolvulus alsinoids, Withania somnifera* and *Matricaria recutita* with the common name of Dwarf Slender Morning Glory, Ashwagandha and Chamomile. The dried powders of all three drugs were purchased from Merlion Naturals, Ahmedabad, India. The powder obtained was characterized and authenticated microscopically in our facility. The powder was ground into a coarse powder with a mortar and pestle before being blended with Carboxymethyl cellulose (CMC) (1.5 %) to create a full suspension for oral dosage at a concentration of 50, 100, 150, 200 mg/kg b.wt of each drug individually. And combinations of the drugs were also given which is listed in Table 1. Diazepam was used as a reference control (Singh et al., 2011).

Animals with ethical statement

The animal experiment was carried out as per the instructions in the protocol number MB/IAEC/2024/02/02 given by the ethical committee of Mass Biotech, Chengalpattu with CCSEA registration number 2084/PO/RcBiBt/S/2019/CCSEA. The study was conducted at the duration of June 2024 to November 2024. Swizz albino mice (male) of weight 40 ± 5g 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-libidum to water (RO water) and a commercially available Rodent pellet diet with 18 percent protein. The conditions were ensured as per CCSEA regulations.

Acute oral toxicity

To study the acute toxicity in animals, OECD 423 test guidelines (TG), was followed. The study was conducted in a female, nulliparous,

Table 1
List of combination groups with the respective treatment and dosage of Combination groups.

Group	Ashwagandha (mg/kg b.wt)	Chamomile (mg/kg b.wt)	Morning Glory (mg/kg b.wt)
Group I	100	100	0
Group II	0	100	100
Group III	100	0	100
Group IV	100	100	100
Group V	100	100	200
Group VI	100	200	200

non-pregnant Swizz albino mice. A single dose of Ashwagandha, Chamomile and morning glory was given to Swiss albino mice. Three animals were used per dose i.e., 5, 50, 300 and 2000 mg/kg. b.wt and all three extracts were performed individually. Clinical observation was done post dosing and at least once a day, up to the end of the day to assess survival, and maintain the general condition. Body weights were recorded before dose administration, on day 7th, and just before the necroscopy (14th day). Animals were euthanized on day 14th and subjected to gross pathological examination was conducted.

Experimental protocol for seizure evaluation

Swiss Albino mice of weight 40 ± 5g were divided into 20 groups randomly with 5 animals in each group, based on their body weight. There are 4 different concentrations in each herb (totally 12 groups), 6 groups for combination of the three herbs, a PTZ control and a Reference control (Diazepam), so totally 20 groups and a total of 100 animals. Table 1 lists the groups and their respective treatment and dosage of drugs in combination.

The table presents the details of six different combination groups used in a study, each with varying dosages of three substances: Ashwagandha**,** Chamomile, and Morning Glory. The dosages are expressed in milligrams per kilogram of body weight (mg/kg b.wt).

The animals were dosed orally daily once for a week. PTZ control animals were given 1.5 % CMC orally, Diazepam was given intraperitoneally (1 mg/kg b.wt (Gowda et al., 2012)) and the rest of the groups were dosed with their respective formulations (Table 1). On the 8th day, all animals were tested for epilepsy. The animals were dosed 30 min before the induction of epilepsy. PTZ (120mg/kg b.wt (Asadi-Shekaari et al., 2014; Johnson et al., 2018)) 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. Every animal was examined individually after administering PTZ for 7 min.

Seizure recording

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 mice was recorded for 7 min 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).

Statistical analysis

The results obtained from the seizure recording were subjected to statistical analysis by using Graph pad Prism 10. The data were compared with 2-way ANOVA for significance (95 % confidence interval)

Results

In acute study conducted as per OECD 423 guidelines, necropsy was conducted on Day 14 and it showed no abnormalities in all groups i.e., upto 2000 mg/kg. b.wt. Histopathology results also reveled normal signs. So it was concluded that, the median lethal dose was 1000 mg/kg. b.wt.

On the eighth day of treatment, PTZ was given to the animals 30 min after dosing, and each animal had a seizure recorded for 7 min. The seizure was evaluated as Latency Period and Seizure Period.

Table 2 presents the effects of various treatments on seizure latency and duration. The PTZ control group shows a relatively short latency (48.4 seconds) and long seizure duration (77.98 seconds). Diazepam, significantly increases latency (189.66 seconds) and completely eliminates seizures. Ashwagandha shows a dose-dependent effect, with higher doses leading to longer latency periods and shorter seizure durations (7.85 ± 15.7 s), particularly at 200 mg/kg, which has the strongest anticonvulsant effect. Chamomile, similarly, demonstrates improved seizure control with higher doses, reducing seizure duration and increasing latency. Morning Glory's effects are also showing that it is dose dependent and 200 mg/kg b.wt dose of morning glory has the lowest seizure period of 6.5 s, suggesting anticonvulsant properties presented in Table 2.

Table 2 presents the effects of different treatments on the latency period and seizure. The treatment groups include, PTZ control, Diazepam, and groups treated with varying doses of Ashwagandha, Chamomile and Morning Glory extract (50 mg/kg to 200 mg/kg body weight). The values are presented as means with their respective standard deviations.

Table 3 **represents the latency and seziure period of the combination groups. Group I** (Ashwagandha and Chamomile) with the latency of 105 s and seizure duration of 26.1 s, wheras, **Group II** (Chamomile and Morning Glory) has a shorter latency (52.5 s) and longer seizure duration (27.92 s), than Group I suggesting that while these two compounds have some antagonistic effect, and so less effective in controlling seizures. **Group III** (Ashwagandha and Morning Glory) shows moderate effects, with a latency of 76.25 s and a seizure period of 20.37 s, indicating that the two compounds together have some anticonvulsant benefits but not as effective as Group I. **Group IV** (all three compounds at 100 mg/kg) shows moderate seizure control, with a latency of 56 s and a seizure period of 17.85 s. **Group V** (with a higher dose of Morning Glory at 200 mg/kg) improves seizure control, reducing the seizure duration to 7.92 s. Finally, **Group VI** (with increased doses of both Chamomile and Morning Glory at 200 mg/kg) demonstrates the most effective anticonvulsant response so far, with the longest latency (168.25 s) which is more than the reference drug Diazepam and the shortest seizure duration (3.82 s), suggesting that higher doses of Chamomile and Morning Glory provide the best seizure control.

Fig. 1 illustrates the graphical representation of Latency period (time between the administration of PTZ and appearance of first jerk) of individual groups (of combination) in seconds.

Fig. 1 illustrates the latency periods across various groups treated with combinations of Ashwagandha, Chamomile, and Morning Glory, compared to PTZ control and Diazepam. The PTZ control group exhibits the shortest latency at 47.4, serving as the baseline. Diazepam significantly increases the latency to 103 ($p < 0.05$). Group 1 shows a latency of 105, which is similar to the effect of Diazepam. Group 6 (Group VI: Ashwagandha 100 mg/kg, Chamomile 200 mg/kg, Morning Glory 200

Table 2
Values of latency and seizure periods of individual groups.

Group	Latency period (s)	Seizure period (s)
PTZ control	48.4 ± 12.34	77.98 ± 19.22
Diazepam	189.66 ± 48.29	0 ± 0
Ashwagandha 50 mg/kg b.wt	65 ± 15.63	36.2 ± 33.55
Ashwagandha 100 mg/kg b.wt	68.5 ± 7.04	43.38 ± 42.87
Ashwagandha 150 mg/kg b.wt	93.4 ± 9.76	11.66 ± 16.44
Ashwagandha 200 mg/kg b.wt	134 ± 40.44	9.85 ± 15.7
Chamomile 50 mg/kg b.wt	107.2 ± 33.84	44.9 ± 31.55
Chamomile 100 mg/kg b.wt	107 ± 38.09	27.2 ± 35.3
Chamomile 150 mg/kg b.wt	117.75 ± 51.03	21.7 ± 29
Chamomile 200 mg/kg b.wt	152 ± 27	7.48 ± 14.02
Morning Glory 50 mg/kg b.wt	69 ± 10.69	29.92 ± 12.92
Morning Glory 100 mg/kg b.wt	57 ± 14.31	17.21 ± 10.58
Morning Glory 150 mg/kg b.wt	128.6 ± 4.27	14.18 ± 3.81
Morning Glory 200 mg/kg b.wt	137.5 ± 95.45	6.5 ± 14.53

Table 3
Values of latency and seizure periods of combination groups.

Group	Latency period (s)	Seizure period (s)
Group I	105 ± 15.87	26.1 ± 3.09
Group II	52.5 ± 8.06	27.92 ± 1.98
Group III	76.25 ± 11.14	20.37 ± 14.75
Group IV	56 ± 12.46	17.85 ± 4.11
Group V	60.5 ± 9.32	7.92 ± 9.22
Group VI	168.25 ± 30.4	3.82 ± 7.65

mg/kg) stands out with the longest latency at 168.25, indicating a highly significant improvement ($p < 0.01$), likely due to synergistic effects. In contrast, Groups 2, 3, 4, and 5 exhibit moderate increases in latency (ranging from 52.5 to 76.25), reflecting lesser efficacy. Overall, Group 6 demonstrates the most pronounced impact on prolonging latency.

Fig. 2 illustrates the graphical representation of seizure period (from the first jerk to the end of postictal depression, which includes clonic, tonic period and postictal depression) of individual groups (of combination) in seconds.

Fig. 2 depicts the seizure period across different groups compared to the PTZ control and Diazepam. The PTZ control group has the longest seizure period at 150.02 s. In contrast, Diazepam completely eliminates seizures (0 seconds). Among the combinations, Group 6 showing the most significant reduction compared to the control ($p < 0.05$). These results suggest a dose-dependent effect, with Group 6 being the most effective in reducing seizure duration.

Discussion

Epilepsy is a neurological disorder characterized by convulsive seizures. Between 50 and 70 million people worldwide are impacted, and safer medications with superior anticonvulsant qualities are greater accessibility are still needs (Yu et al., 2019). Numerous medications have been used to treat epilepsy. These medications have a number of adverse effects that can occasionally be fatal. Science more than 25 % of patients taking antiepileptic medications are resistant to conventional drug therapies, it is imperative to find novel medications, particularly made from natural ingredients, that will work well few or no adverse effects (Ugwah-Oguejiofor et al., 2023). Anticonvulsant medication screening uses PTZ, a convulsant agent. By blocking the Gama amino-butyric acid (GABA) pathway at GABA-A receptors, it causes seizures. GABA is a key inhibitory neurotransmitter implicated in epilepsy. By activation the N-methyl-D-aspartate (NMDA) receptor, it also engages in glutamatergic mechanisms, which seem to play a role in the start and spread of PTZ-induced seizures (Amabeoku, 1999; Kwan and Brodie, 2006). Pilocarpine is also used to induce seizures in mice and rats (Bezza et al., 2019).

Molecular docking of phytochemicals in Ashwagandha revels, hygrine, tropine and withasomnine form W. somnifera acts as a GABA-A receptor agonists which eventually eliminates the effect if PTZ (Alam et al., 2024). Methanolic extract and aqueous of Withinia sominifera generates inward current which leads to agonist activity on GABA-A receptors (Candelario et al., 2015). Certain natural and synthesized flavonoids have been shown in some studies to exhibit anxiolytic effects in rats, and it has been discovered that these substances work by binding to central benzodiazepine receptors, which are also associated with M. recrutita (Heidari et al., 2009) (-)-α-bisabolol (BSB), is a levomenol and an unsaturated sesquiterpene alcohol present in *M.recrutita* revels reduced level of TNF-α, IL-1β, and MDA oxidative markers which is a sign of controlling seizures (Nazarinia et al., 2023).

(K et al., 2023) Revels the presence of phytochemicals in *M. recrutita*. Various components of alkaloids have been reported to possess therapeutic potentials such as vinblastine and camptothecin (anticancer), morphine and codeine (analgesics), ephedrine (nervous system stimulant), and colchicine (anti-inflammatory) in *M. recrutita* (Pallardy, 2008). The presence of these phytochemicals in *E. alsinoides* may be responsible for some of its reported biological effects (antioxidants, anti-depressant, neuropharmacological effects, cardiovascular activity, etc (Sethiya et al., 2019).

The present study evaluated the anticonvulsant activities of the

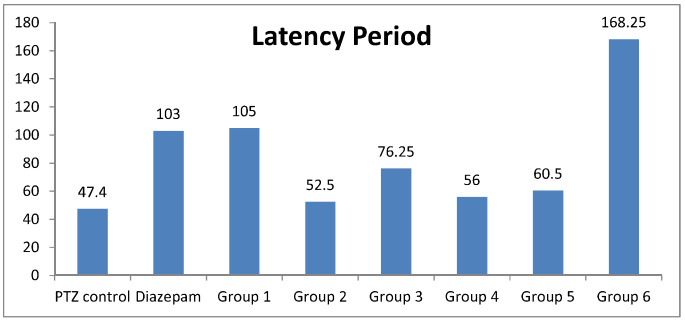

Fig. 1. Latency period of individual groups.

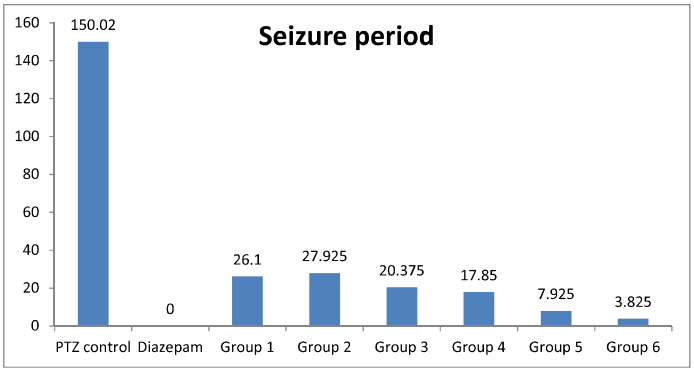

Fig. 2. Seizure period of individual groups.

individual and combined form of Ashwagandha, Morning Glory and chamomile in chemically induced mice. The extracts showed dose dependent protection in PTZ-induced seizure test in mice. Dose of PTZ varies from 30 to 120 mg/kg. b.wt to induce seizures (Mante et al., 2013) shows significant difference in the stages of seizures (Shrivastava et al., 2022; Ugwah-Oguejiofor et al., 2023). Upon experimenting, we finalized the dose of 120 mg/kg. b.wt of PTZ. In (Ugwah-Oguejiofor et al., 2023) they have used aqueous extract of Carallum adalzielii on PTZ induced seizure where the seizure period of 500 mg/kg of extract was 15.5 ± 6.8 but here in all three extracts, 200 mg/kg of extract had seizure period below 10 s. And in combination, the seizure period was reduced up to 3 s (Table 3). Aqueous seed extract of Ashwagandha was evaluated against Pilocarpine induced epilepsy in rats, which revealed a positive effect by reducing the hippocampus serotonin and dopamine levels equal to the reference control and reduces the seizure (Sayyar et al., 2018). Effect of Withania somnifera (Ws) on PTZ-seizue threshold showed only at 120 mg/kg of PTZ was able to induce tonic seizure in Ws (200 mg/kg) treated mice (Kulkarni et al., 2008). (Heidari et al., 2009) used Picrotoxin to induce seizure and evaluated it with Matricaria recutita extract. Seizure latency of Picrotoxin was more than 120s and seizure period lasts more than 50 min. This is due to usage of Picrotoxin to induce seizure but Matricaria recutita extract reduced the seizure period to 15 min. Ethyl acetate extract of Matricaria recutita against strychnine induced seizures, showed the onset of seizure was more than 200 s in test drug (25 mg/kg) (Hamad et al., 2019). In extract of Punica granatum, and our 3 extracts have almost same effect on latency period of around 150 s in the dose 200 mg/kg. b.wt (G.L. et al., 2016). In (Mante et al., 2013) where they challenged mice with PTZ of dose 85 mg/kg b.wt, control group's seizure duration lasted for more than 1000 s and the maximum effect of Antiaris toxicaria was more than 400 s which is way more than results obtained from this combination. Methanolic extract of Evolvulus alsinoides in PTZ-induced seizure in mice had latency period of 983 s in 200 mg/kg b.wt dose which is way more than the results here and they have also evaluated with Maximal electric shock seizure method where the Tonic Clonic seizure time for 200 mg/kg b.wt was 12.4 s but here it was 6.5 s (Pharm et al., 2013). It was also used to study nootropic activity in scopolamine-induced amnesia in mice which showed promising results (nootropic).

We have discussed about the effect of Evolvulus alsinoides, Withania somnifera and Matricaria recutita in our previous publications (Venkatesan et al., 2024a, b).

Conclusion

The study demonstrates that *Evolvulus alsinoides* (Dwarf Slender Morning Glory) *Withania somnifera* (Ashwagandha) and *Matricaria recutita* (Chamomile) possesses significant anticonvulsant properties in a PTZ-induced seizure model in mice. The results demonstrate the extrats of *Evolvulus alsinoides, Withania somnifera* and *Matricaria recutita* individually and in combination has a synergic effect and acts as a strong herbal treatment for epilepsy in the dose of Ashwagandha 100 mg/kg. b.wt, Chamomile 200 mg/kg.b.wt and Morning glory 200 mg/kg. b.wt providing a healthy substitute for conventional antiepileptic medications with a latency and seizure period of 168.25 s and 3.2 s respectively.

Funding

Mass Biotech Private Limited, Chennai, Tamilnadu, India.

Ethical approval

The animal experiment was carried out as per the instructions in the protocol number MB/IAEC/2024/02/02 given by the ethical committee of Mass Biotech, Chengalpattu with CCSEA registration number 2084/PO/RcBiBt/S/2019/CCSEA

CRediT authorship contribution statement

P.S. Venkatesan: Writing – review & editing. **S. Sundaresan:** Writing – original draft, Investigation. **M. Eswarya:** Supervision, Methodology. **M. Madhavaselvi:** Visualization, Data curation. **R. Renuka:** Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Ms Nadhiya for taking care of the animals throughout the experiment. We also thank Ms. Sathya for maintaining the animal house. We thank RR histology services, Hyderabad who helped us with the histopathology results.

References

Adiga, S.H., Adiga, R.S., Bhat, K.M.R., Upadhya, D., 2024. Ayurveda therapy in the management of epilepsy. Epilepsy Behav. 159. https://doi.org/10.1016/j.yebeh.2024.110026.

Akyüz, E., Köklü, B., Ozenen, C., Arulsamy, A., Shaikh, Mohd.F., 2021. Elucidating the potential side effects of current anti-seizure drugs for epilepsy. Curr. Neuropharmacol. 19 (11), 1865–1883. https://doi.org/10.2174/1570159X19666210826125341.

Alam, M., Abbas, K., Iram, F., Raza, M.T., Mustafa, M., Zehra, Z., 2024. Molecular docking and dynamics studies of *withania somnifera* derived compounds as GABA-A receptor modulators for insomnia. Chronobiol. Med. 6 (2), 77–86. https://doi.org/10.33069/CIM.2024.0010.

Al-Snafi, Ali Esmail, Hasham, Lawahidh Fali, 2023. Bioactive constituents and pharmacological importance of Matricaria chamomilla: a recent review. GSC Biol. Pharmaceut. Sci. 22 (2), 079–098. https://doi.org/10.30574/GSCBPS.2023.22.2.0477.

Alqahtani, A.S., Ullah, R., Shahat, A.A., 2022. Bioactive constituents and toxicological evaluation of selected antidiabetic medicinal plants of Saudi Arabia. Evid.-Based Compl. Altern. Med. 2022. https://doi.org/10.1155/2022/7123521.

Amabeoku, G.J., 1999. Gamma-aminobutyric acid and glutamic acid receptors may mediate theophylline-induced seizures in mice. General. Pharmacol.: Vascul. Syst. 32 (3), 365–372. https://doi.org/10.1016/S0306-3623(98)00201-8.

Asadi-Shekaari, M., Eslami, A., Kalantaripour, T., Joukar, S., 2014. Potential mechanisms involved in the anticonvulsant effect of walnut extract on pentylenetetrazole-induced seizure. Med. Principles Pract. : Int. J. Kuwait Univ., Health Sci. Centre 23. https://doi.org/10.1159/000365759.

Auxtero, M.D., Chalante, S., Abade, M.R., Jorge, R., Fernandes, A.I., 2021. Potential herb–drug interactions in the management of age-related cognitive dysfunction. Pharmaceutics 13 (1), 1–70. https://doi.org/10.3390/PHARMACEUTICS13010124.

Awere, C.O., Anadebe, V.C., Kasinathan, R., Muthuramalingam, P., Manikandan, R., 2025. State of the art progress of Evolvulus alsinoides in pharmacological activity and plant tissue culture: A potent Chinese medicinal plant. Pharmacolog. Res.-Modern Chinese Med. 14, 100586. https://doi.org/10.1016/J.PRMCM.2025.100586.

Bastos, F., Cross, J.H., 2020. Epilepsy. Handbook Clin. Neurol. 174, 137–158. https://doi.org/10.1016/B978-0-444-64148-9.00011-9.

Bezza, K., Gabbas, Z.El, Laadraoui, J., Laaradia, M.A., Oufquir, S., Chait, A., 2019. Ameliorative potential of Anacyclus pyrethrum extract in generalized seizures in rat: Possible cholinergic mediated mechanism. Bangladesh J. Pharmacol. 14 (4), 188–195. https://doi.org/10.3329/BJP.V14I4.40537.

Candelario, M., Cuellar, E., Reyes-Ruiz, J.M., Darabedian, N., Feimeng, Z., Miledi, R., Russo-Neustadt, A., Limon, A., 2015. Direct evidence for GABAergic activity of Withania somnifera on mammalian ionotropic GABAA and GABAρ receptors. J. Ethnopharmacol. 171, 264–272. https://doi.org/10.1016/J.JEP.2015.05.058.

shastry, G.L.V., Marikunte, V., Prasad, N.B.L., Godavarthi, A., 2016. Evaluation of anti-epileptic activity of leaf extracts of punica granatum on experimental models of epilepsy in mice. J. Int. Ethnopharmacol. 6, 1. https://doi.org/10.5455/jice.20160904102857.

Gomathi, D., Kalaiselvi, M., Ravikumar, G., Devaki, K., Uma, C., 2015. GC–MS analysis of bioactive compounds from the whole plant ethanolic extract of Evolvulus alsinoides (L.) L. J. Food Sci. Technol. 52 (2), 1212–1217. https://doi.org/10.1007/S13197-013-1105-9.

Gowda, G., Das, K., Bhosle, V., Einstein, J., K, B., 2012. Evaluation of anticonvulsant activity of ethanolic leaves extract of Desmodium triflorum in mice. Revista Brasileira de Farmacognosia 22, 649–656. https://doi.org/10.1590/S0102-695X2012005000019.

Hakami, T., 2021. Neuropharmacology of antiseizure drugs. Neuropsychopharmacol. Reports 41 (3), 336–351. https://doi.org/10.1002/NPR2.12196.

Hamad, M., Sulaiman, A., Numan, I., Al, S., Abdulwahab, D., & Razak, A. (2019). *Study of anticonvulsant effect of ethyl acetate fraction of matricaria recutita extract in mice*. https://doi.org/10.13140/RG.2.2.10693.29925.

Hashemi, P., Fahanik Babaei, J., Vazifekhah, S., Nikbakht, F., 2019. Evaluation of the neuroprotective, anticonvulsant, and cognition-improvement effects of apigenin in temporal lobe epilepsy: Involvement of the mitochondrial apoptotic pathway. Iranian J. Basic Med. Sci. 22 (7), 752–758. https://doi.org/10.22038/IJBMS.2019.33892.8064.

Heidari, M.R., Dadollahi, Z., Mehrabani, M., Mehrabi, H., Pourzadeh-Hosseini, M., Behravan, E., Etemad, L., 2009. Study of antiseizure effects of Matricaria recutita extract in mice. Ann. N.Y. Acad. Sci. 1171, 300–304. https://doi.org/10.1111/J.1749-6632.2009.04917.X.

Johnson, W., Boyer, I., Bergfeld, W.F., Belsito, D.V, Hill, R.A., Klaassen, C.D., Liebler, D. C., Marks, J.G., Shank, R.C., Slaga, T.J., Snyder, P.W., Gill, L.J., Heldreth, B., 2018. Amended safety assessment of chamomilla recutita-derived ingredients as used in cosmetics. Int. J. Toxicol. 37 (3_suppl), 51S–79S. https://doi.org/10.1177/1091581818801814.

K, N., R, S., PP, S., M, R., V, B.C., 2023. Biochemical analysis of methanolic extract from Evolvulus alsinoides. Bioinformation 19 (12), 1173–1178. https://doi.org/10.6026/973206300191173.

Kondziella, D., Bidar, A., Urfjell, B., Sletvold, O., Sonnewald, U., 2002. The pentylenetetrazole-kindling model of epilepsy in SAMP8 mice: Behavior and metabolism. Neurochem. Int. 40 (5), 413–418. https://doi.org/10.1016/S0197-0186(01)00104-8.

Kulkarni, S., Akula, K., Dhir, A., 2008. Effect of withania somnifera dunal root extract against pentylenetetrazol seizure threshold in mice: possible involvement of GABAergic system. Indian J. Exp. Biol. 46, 465–469.

Kwan, P., Brodie, M.J., 2006. Refractory epilepsy: mechanisms and solutions. Expert. Rev. Neurother. 6 (3), 397–406. https://doi.org/10.1586/14737175.6.3.397.

Mante, P.K., Adongo, D.W., Woode, E., Kukuia, K.K.E., Ameyaw, E.O., 2013. Anticonvulsant effect of antiaris toxicaria (Pers.) Lesch. (Moraceae) aqueous extract in rodents. ISRN Pharmacol. 2013, 1–9. https://doi.org/10.1155/2013/519208.

Mehla, J., Gupta, P., Pahuja, M., Diwan, D., Diksha, D., 2020. Indian medicinal herbs and formulations for Alzheimer's disease, from traditional knowledge to scientific assessment. Brain Sci. 10 (12), 1–31. https://doi.org/10.3390/BRAINSCI10120964.

Moghimi, S., Harini, B.P., 2022. A comparative study of the efficiency of Withania somnifera and carbamazepine on lifespan, reproduction and epileptic phenotype - A study in Drosophila paralytic mutant. J. Ayurveda Integ. Med. 13 (2). https://doi.org/10.1016/J.JAIM.2021.11.002.

Nazarinia, D., Moslehi, A., Hashemi, P., 2023. (-)-α-bisabolol exerts neuroprotective effects against pentylenetetrazole-induced seizures in rats by targeting inflammation and oxidative stress. Physiol. Behav. 272. https://doi.org/10.1016/j.physbeh.2023.114351.

Padi, P.M., Adetunji, T.L., Unuofin, J.O., Mchunu, C.N., Ntuli, N.R., Siebert, F., 2022. Phytochemical, antioxidant, and functional group analyses of South African Evolvulus alsinoides (L.) L. S. Afr. J. Bot. 149, 170–177. https://doi.org/10.1016/J.SAJB.2022.06.005.

Pallardy, S.G., 2008. Nitrogen Metabolism. Physiol. Woody Plants 233–254. https://doi.org/10.1016/B978-012088765-1.50010-5.

Pharm, Kabir, A., Ugwah-Oguejiofor, C., N, U., B, A., Abdulkadir, R., 2013. Evaluation of the Anticonvulsant Effect of the Methanol Extract of Evolvulus Alsinoides in Mice, 2.

Sah, A., Naseef, P., Kuruniyan, M., Jain, G., Zakir, F., Aggarwal, G., 2022. A comprehensive study of therapeutic applications of chamomile. Pharmaceuticals 15, 1284. https://doi.org/10.3390/ph15101284.

Sahu, P., Gupta, S., 2014. Medicinal Plants Of Morning Glory: Convolvulaceae Juss. Of Central India (Madhya Pradesh & Chhattishgarh). Biolife 2.

Saleem, S., Muhammad, G., Hussain, M.A, Altaf, M., Abbas Bukhari, S.N., 2020. Withania somnifera L.: Insights into the phytochemical profile, therapeutic potential, clinical trials, and future prospective. Iranian J. Basic Med. Sci. 23 (12), 1501–1526. https://doi.org/10.22038/IJBMS.2020.44254.10378.

Sayyar, Z., Yazdinezhad, A., Hassan, M., Jafari Anarkooli, I., 2018. Protective effect of *matricaria chamomilla* ethanolic extract on hippocampal neuron damage in rats exposed to formaldehyde. Oxid. Med. Cell. Long. 2018, 6414317. https://doi.org/10.1155/2018/6414317.

Sethiya, N., Nahata, A., Singh, P., Mishra, S., 2019. Neuropharmacological evaluation on four traditional herbs used as nervine tonic and commonly available as Shankhpushpi in India. J. Ayurveda Integ. Med. 10, 25–31. https://doi.org/10.1016/j.jaim.2017.08.012.

Shimada, T., Yamagata, K., 2018. Pentylenetetrazole-Induced Kindling Mouse Model. J. Visualized Experim. : JoVE 2018 (136). https://doi.org/10.3791/56573.

Shrivastava, A., Gupta, J.K., Goyal, M.K., 2022. Potential efficacy of ocimum sanctum hydro-alcoholic leaf extract as an adjuvant role with phenobarbital: acute models of epilepsy on mice. Int. J. Nutrit., Pharmacol., Neurolog. Diseases 12 (3), 134–141. https://doi.org/10.4103/IJNPND.IJNPND_9_22.

Singh, N., Bhalla, M., de Jager, P., Gilca, M., 2011. An Overview on Ashwagandha: A Rasayana (Rejuvenator) of Ayurveda. Afr. J. Tradit., Complem. Alternative Med. : AJTCAM /Afr. Networks Ethnomed. 8, 208–213. https://doi.org/10.4314/ajtcam.v8i5S.9.

Tanna, I., Aghera, H., Bk, A., Chandola, H., 2012. Protective role of Ashwagandharishta and flax seed oil against maximal electroshock induced seizures in albino rats. Ayu 33, 114–118. https://doi.org/10.4103/0974-8520.100327.

Ugwah-Oguejiofor, C.J., Amuda, M.B., Abubakar, K., Ugwah, O.M., Ofokansi, M.N., Mshelia, H.E., 2023. An experimental evaluation of anticonvulsant activity of aqueous extract of Caralluma dalzielii N.E. Brown. Phytomedicine Plus 3 (1), 100401. https://doi.org/10.1016/J.PHYPLU.2022.100401.

Van Erum, J., Van Dam, D., De Deyn, P.P., 2019. PTZ-induced seizures in mice require a revised Racine scale. Epilepsy Behav. : E&B 95, 51–55. https://doi.org/10.1016/J.YEBEH.2019.02.029.

Venkatesan, Dr.P.S., Eswarya, M., Madhavaselvi, M., 2024a. Evaluation of anti-epileptic properties of ashwagandha and chamomile by PTZ-induced mouse model. Int. J. Pharma Bio Sci. 15 (3), 16–22. https://doi.org/10.22376/IJPBS.2024.15.3.P16-22.

Venkatesan, P.S., Eswarya, M., Madhavaselvi, M., 2024b. Evaluation of anti-epileptic properties of Evolvulus alsinoides by pentylenetetrazole-induced mouse model. Int. J. Basic Clin. Pharmacol. 13 (5), 673–678. https://doi.org/10.18203/2319-2003.IJBCP20242427.

Wagh, S., Dhuri, K., 2023. Vishnukranta (Evolvulus alsinoides Linn.) : a clinical drug review. J. Ayurv. Integ. Med. Sci. 8, 149–152. https://doi.org/10.21760/jaims.8.10.22.

Yu, X., Guan, Q., Wang, Y., Shen, H., Zhai, L., Lu, X., Jin, Y., 2019. Anticonvulsant and anti-apoptosis effects of salvianolic acid B on pentylenetetrazole-kindled rats via AKT/CREB/BDNF signaling. Epilepsy Res. 154, 90–96. https://doi.org/10.1016/J.EPLEPSYRES.2019.05.007.

Zolkowska, D., Banks, C., Dhir, A., Inceoglu, B., Sanborn, J., Mccoy, M., Bruun, D., Hammock, B., Lein, P., Rogawski, M., 2012. Characterization of Seizures Induced by Acute and Repeated Exposure to Tetramethylenedisulfotetramine. J. Pharmacol. Exp. Ther. 341, 435–446. https://doi.org/10.1124/jpet.111.190579.