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Original Research Article

Evaluation of anti-epileptic properties of *Evolvulus alsinoides* by pentylenetetrazole-induced mouse model

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ABSTRACT

Background: A common neurological condition that affects people of all ages, epilepsy is defined by recurring seizures that have serious negative effects on the nervous system, cognition, psychology, and social interactions. Anti-epileptic 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), this study examines the anticonvulsant efficacy of a traditional medicinal plant called *Evolvulus alsinoides*, often known as dwarf slender morning glory. The herb was mixed with 1.5% CMC and given to mice with various dosage levels.
Results: The effects of various doses of morning glory extract (50 mg/kg to 400 mg/kg body weight) on latency period and seizure duration were compared to those of a control group and a diazepam-treated group. In latency period, 300 mg/kg. b. wt group showed a long latency period of 222.5±47.68 ($p<0.05$) when comparing to other groups. Morning glory of dose 400 mg/kg b. wt eliminated tonic-clonic seizures in all animals ($p<0.001$).
Conclusions: These findings suggest that *Evolvulus alsinoides* has anti-epileptic properties in PTZ-induced mice model.

Keywords: *Evlolvulus alsinoides*, PTZ, Diazepam, Epilepsy, Mouse model and morning glory

INTRODUCTION

Epilepsy is one of the most common neurological diseases which affects people of all age. A persistent propensity to have seizures and the neurobiological, cognitive, psychological, and social ramifications of seizure recurrences are the hallmarks of epilepsy, a brain illness.[1] Although seizure cycles have been observed since antiquity, current technology advancements have made it possible to quantitatively define seizure cycles using direct brain recordings for the first time.[2] Numerous medications are under investigation for the treatment of epilepsy; several of these focus on pathophysiological pathways that were previously overlooked but have a good efficacy profile and mild to moderate adverse events.[3] Many individuals are concerned about the side effects associated with epilepsy medications, as these can significantly impact their quality of life. Anti-epilepsy drugs may cause undesirable side effects in some patients, which can lead to difficulties in adhering to their prescribed treatment regimen.[4] Tiredness, upset stomach, dizziness, and blurred vision are frequent side effects that typically occur in the first few weeks of taking medication for seizures. These drugs may also cause fatigue, nausea, urinary retention and sexual dysfunction.[5] These days, herbal remedies are the most common complementary and alternative medicine approach. They are crucial to the treatment of epileptic seizures or side effects from antiepileptic

pharmaceuticals.[6] To avoid these kinds of side-effects, people prefer herbal medicines not only for epilepsy, but also for all kinds of disorders and diseases.

In India, *Evolvulus alsinoides* is referred to as Vishnukranta and commonly known as dwarf slender morning glory.[7] It is a significant medicinal plant that thrives in grassy, open areas practically everywhere in India and other subtropical nations.[8] *Evolvulus alsinoides* L., a member of the *Convolvulaceae* family, is a type of weed native to tropical and subtropical swamp regions worldwide, primarily found in East Asia.[9] This perennial herb is prostrate and has a short woody rootstock that branches outward. Branches are numerous, yearly, more than 30 centimeters, and frequently erect with long hairs. The leaves are sessile, tiny, elliptic, sharp, and heavily hairy. The flowers are lonely and blue.[10]

In Ayurveda, the entire plant is used to treat amnesia, asthma, and neurological illnesses.[11,12] It also has antispasmodic, anti-haemorrhagic, antioxidant, and anti-inflammatory properties.[13] This herb is used in a number of nootropic medications. The current study uses seizure models caused by PTZ to examine the potential anticonvulsant efficacy of *Evolvulus alsinoides*.

METHODS

Preparation of herbal drugs

The herbal drug used is *Evolvlus alsinoids* with the common name of dwarf slender morning glory. The dried powder of morning glory was 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 CMC (1.5%) to create a full suspension for oral dosage at a concentration of 50, 100, 200, 300, and 400 mg/kg b. wt of morning glory. Diazepam was utilized as a reference control.[14]

Animals with ethical statement

The animal experiment was carried out as per the instructions in the protocol number MB/IAEC/2024/01/04 given by the ethical committee of Mass Biotech, Chengalpattu with CPCSEA registration number 2084/PO/RcBt/S/19/CPCSEA. The study was conducted at the duration of December 2023 to June 2023. Swizz albino mice (male) of weight 40±5 gm 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 12 hr light/12 hr 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 CPCSEA regulations.

Experimental protocol

Swiss Albino mice of weight 40±5 g were divided into 6 groups randomly based on their body weight. Table 1 lists the groups and their respective treatment and dosage of drugs.

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

Groups	Treatment	Dose (mg/kg b. wt)	Number of animals
Group I	PTZ control	120	5
Group II	Diazepam+ PTZ	1	5
Group III	Morning glory +PTZ	50	5
Group IV	Morning glory + PTZ	100	5
Group V	Morning glory PTZ	200	5
Group VI	Morning glory + PTZ	300	5
Group VII	Morning glory + PTZ	400	5

Table 1 illustrates the different groups used in the study. Also, it has information on the different treatments listing the dosage of the respective. PTZ was administered in a dose of 120 mg/kg b.wt and diazepam (reference control) of 1 mg/kg b. wt. Treatments including morning glory of concentration of 50, 100, 200, 300, 400 mg/kg b. wt. Acute toxicity study was conducted and based on which the concentration of herbal drugs were chosen. Each group consists of 5 animals.

The animals were dosed orally for a week. PTZ control animals were given 1.5% CMC orally, diazepam was given intraperitoneally (1 mg/kg b. wt) and the rest of the groups were dosed with their respective formulations (Table 1).[15] On the 8th day, all animals were tested for epilepsy. The animals were dosed 30 minutes before the induction of epilepsy. PTZ (120 mg/kg b. wt) is used for the induction of seizures.[16,17] PTZ was purchased from SRL chemicals. It is 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), 2) Clonic period (period of repeated clonic jerks of the forelimbs and hindlimbs with loss of the righting reflex), 3) Tonic period (falling on the side followed by

forelimb and hindlimb tonic contractions), 4) Postictal depression (the period between the end of tonic seizure to the normal stage of mice).[14,18,19] 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 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).

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

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

The latency period information for PTZ control, diazepam, and different morning glory extract dosages show how well these medications work in delaying the onset of seizures which is showed in Figure 1. With latency duration of 48.4 s, the PTZ control group indicates how quickly seizures can start when left untreated. The standard anticonvulsant Diazepam significantly prolongs this duration to 189.67 s. The morning glory extract shows a dose-dependent response in increasing latency periods. The delay period slightly increases to 66 s at 50 mg/kg, suggesting some anticonvulsant action. Interestingly, the 100 mg/kg dose results in a latency of 59, slightly less effective than the 50 mg/kg dose, which suggests a non-linear response at these lower doses.

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

Groups	Latency period (s)	Seizure period (s)
PTZ control	48.4±12.34	77.98±19.22
Diazepam	189.66±48.29	0±0
Morning glory 50 mg/kg b. wt	66±15.53	27.98±16.24
Morning glory 100 mg/kg b. wt	59±13.11	17.38±16.59
Morning glory 200 mg/kg b. wt	137.5±95.45	6.5±14.53
Morning glory 300 mg/kg b. wt	222.5±47.68	4.82±10.77
Morning glory 400 mg/kg b. wt	205±28	0

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 morning glory extract (50 mg/kg to 400 mg/kg body weight). The values are presented as means with their respective standard deviations.

On the other hand, the latency duration dramatically increases to 137.5 s at 200 mg/kg, indicating a significant improvement in delaying the start of seizures. The greatest measured delay period of 222.5 s ($p<0.05$), suggesting a potent anticonvulsant action, is shown at 300 mg/kg, which also shows the most noticeable effect. Though still much greater than the control and lower dosages, the 400 mg/kg dose causes a modest drop-in latency time to 205 s, making it marginally less effective than the 300 mg/kg dose. This suggests that while higher doses of morning glory extract are effective, there may be an optimal dose range around 300 mg/kg for maximal efficacy.

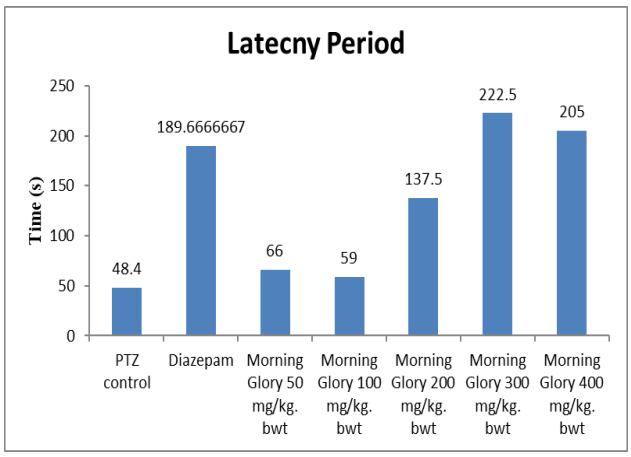

Figure 1: Latency period of individual groups.

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

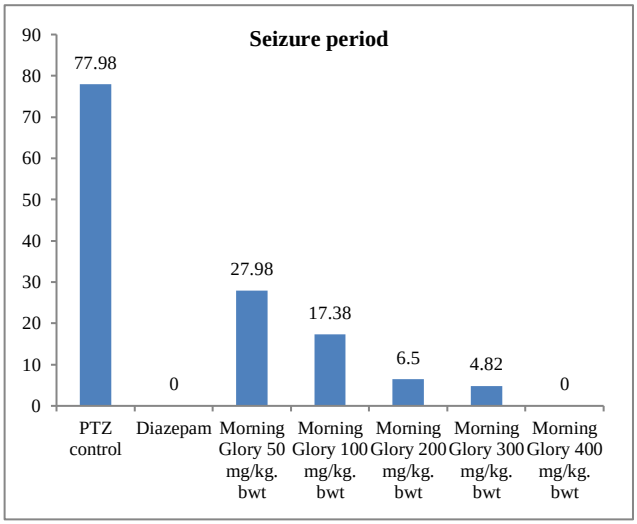

Figure 2: Seizure period of individual groups.

Speaking of the seizure period, as in Figure 2 the PTZ control group, with a seizure period of 77.98 seconds. Diazepam completely eliminates seizures, while morning glory extract shows a dose-dependent reduction. At 50 ($p<0.01$), 100, 200 mg/kg ($p<0.001$), the seizure period drops eventually and in 300 mg/kg ($p<0.001$), it almost eliminates seizures. Similar to the effects of diazepam, the maximum dose of 400 mg/kg ($p<0.001$) fully eliminates the seizure period, suggesting that this dosage of morning glory extract can completely prevent seizures. Morning glory extract has the potential to be a powerful anticonvulsant, as seen by the steady reduction in seizure length that occurs with increasing doses; at higher doses, the extract has efficacy comparable to that of diazepam.

Figure 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 in seconds. It is evident that morning glory of dose 400 mg/kg. b. wt blocks the seizure like the reference compound diazepam.

DISCUSSION

One of the most prevalent and long-lasting neurological conditions, epilepsy affects about 50 million individuals globally.[20] Even with incredible advancements in research and the creation of potent pharmacotherapies, antiepileptic medications are unable to control clinical seizures in 20-30% of cases.[21] Apart from common physical side effects, these anti-epilepsy drugs reduce the excitability of brain nerve cells, which has an impact on regular function. Cognitive issues can result from side effects of anti-epilepsy drugs and are challenges related to thinking, remembering, paying attention or concentrating, and finding the proper words. One of the most alluring sources of novel medications is plant extracts, and studies on their efficacy in treating epilepsy have produced encouraging results. There are many research studies proving herbal compounds having anti-epileptic properties. Some of them include *Antiaris toxicaria*, *Punica granatum*, *Calotropis procera*, *Pseudospondias microcarpa*, *Cissus quadrangularis*, *Albizia adianthifolia*, *Mallotus oppositifolius*, *Withania somnifera* and *Matricaria recutita*.[22-30] In rodents, epilepsy is chemically induced by PTZ, kainic acid, and pilocarpine.[31] PTZ is an antagonist of the gamma aminobutyric acid (GABA)-A receptor. PTZ increases neuronal activity by inhibiting the function of inhibitory synapses. In animals, this regulation results in generalized seizures.[32,33] In this study, they have used PTZ of dose 100 mg/kg b. wt but here we have used PTZ of dosage 120 mg/kg. b. wt which was comfortable for inducing clonic-tonic seizures.[34] We have already performed this anti-epileptic studies in *Withania somnifera* (Ashwagandha) and *Matricaria recutita* (Chamomile) of only one dose (100 mg/kg b. wt) and performed a combination of both of total dose 100 mg/kg. b. wt.[35] The lethal dose (LD_{50}) of *Evolvulus alsinoides* was reported as 3312 mg/kg. b. wt.[36] Morning Glory gives full protection against PTZ induced seizures in 400 mg/kg. b. wt. When comparing with other herbal extracts, *Desmodium triflorum* only extends the latency period even in a dose of 800 mg/kg. b. wt.[15] In the seizure period recorded was 5.5 s in which they have used 400 mg/kg. b. wt Aloe vera extract.[37] But here, in 400 mg/kg. b. wt, there were no clonic-tonic seizures observed. And also, PTZ used in was 60 mg/kg. b. wt which is less than what we have used here.[37] We have performed the same study in *Withania somnifera* and *Matricaria recutita* in mouse model which is under publication.[35] Here we have only performed only PTZ-mice model. Results would me more promising if it is performed in various epilepsy models and in higher species. This study reveals the anti-epileptic property of *Evolvulus alsinoides* against PTZ-induced mice model. Further, this study will be uplifted by testing mixture of herbs in animal models.

CONCLUSION

The study demonstrates that *Evolvulus alsinoides* (Dwarf slender morning glory) possesses significant anticonvulsant properties in a PTZ-induced seizure model in mice. Similar to the common anticonvulsant diazepam, the extract exhibited a dose-dependent increase in latency periods and a decrease in seizure durations, with the highest dosages (300 mg/kg and 400 mg/kg) successfully preventing seizures. These results demonstrate the potential of *Evolvulus alsinoides* as a strong herbal treatment for epilepsy, providing a healthy substitute for conventional anti-epileptic medications.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee of Mass Biotech, Chengalpattu, Protocol number MB/IAEC/2024/01/04 with CPCSEA registration number 2084/PO/RcBt/S/19/CPCSEA

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