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Original Article

In vivo and *in vitro* Comparative Evaluation of *Delphinium denudatum* and *Amaranthus spinosus* for Anticonvulsant Activity

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ABSTRACT

Introduction: 50 million people worldwide are affected by epilepsy, and a significant proportion suffer from side effects or respond poorly to drugs. With an established use in neurology, *Delphinium denudatum* (DDE) and *Amaranthus spinosus* (ASE) still need further studies on their anticonvulsant abilities. **Materials and Methods:** Hydroethanolic extracts were prepared from DDE roots and ASE leaves. Toxicity was evaluated in albino Wistar rats. Anticonvulsant activity was assessed in Swiss albino mice using the Maximal Electroshock Seizure and Pentylenetetrazole models. GABA levels were analyzed via paper chromatography post-treatment. Doses of 200 and 400 mg/kg were administered. In C1, mice received 100 mg/kg extract and 200 mg/kg phenibut; Diazepam served as the standard in the control group. **Results:** No toxicity or mortality was observed up to 2000 mg/kg. In the MES model, 400 mg/kg of DDE and ASE extracts significantly reduced tonic hind limb extension ($p<0.05$), with Combination C2 being the most effective. Both extracts (200 and 400 mg/kg) suppressed PTZ-induced seizures and reduced convulsion duration. Combination C2 improved survival and restored GABA levels close to normal ($p<0.01$), comparable to Diazepam. **Discussion:** DDE extract had powerful anticonvulsant effects that may result from GABA and sodium channel activity, whereas ASE extract protected the brain by being an antioxidant. By combining different plant extracts, both therapies outlined the promising effects of natural anticonvulsants. **Conclusion:** The study concludes that DDE and ASE, especially in combination, exhibit significant anticonvulsant activity, supporting their potential as natural alternatives for epilepsy management.

Keywords: *Delphinium denudatum*, *Amaranthus spinosus*, Anticonvulsant activity, Epilepsy, GABA modulation, PTZ and MES models.

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INTRODUCTION

Epilepsy manifests as a worldwide chronic neurological condition that produces recurrent seizures, which impact 50 million individuals globally and primarily affects 80% of individuals from low- and middle-income countries where access to contemporary Antiepileptic Drugs (AEDs) remains restricted.[1] About 30% of patients demonstrate non-response to current treatments, and many face severe side effects, including cognitive difficulties, liver damage, and problems with fetal development from using traditional AED medicines such as phenytoin, valproate, and carbamazepine.[2] This therapeutic gap has intensified the search for novel anticonvulsant agents, particularly from plant-derived sources, which historically account for 40% of modern pharmaceuticals and offer potential advantages in safety, accessibility, and multi-target mechanisms. Among the myriad botanicals under investigation,*Delphinium denudatum* (DDE) and *Amaranthus spinosus* (ASE) emerge as compelling candidates, though their anticonvulsant profiles and mechanisms remain underexplored in comparative contexts.[3]

DDE, a perennial herbaceous plant native to the Himalayan region, holds a venerable position in Ayurvedic and Unani medicine, where its roots have been used to treat mental illnesses for hundreds of years, including epilepsy, hysteria, and sciatica. Modern phytochemical analyses reveal a rich profile of norditerpenoid alkaloids, particularly delphinine, delsoline, and denudatine, that exhibit pronounced neuropharmacological activity.[4] Preclinical research shows that its aqueous-alcoholic extract works very well in models of Maximum Electroshock Seizure (MES) and pentylenetetrazole induced seizures, showing 100% protection against tonic hindlimb extensions at 600 mg/

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kg doses, comparable to phenytoin.[5] The FS-1 subfraction of its methanolic extract further exhibits dose-dependent suppression of GABA transaminase activity (IC_{50}=18.2 µg/mL) and glutamate decarboxylase modulation, suggesting dual modulation of excitatory and inhibitory neurotransmission. Crucially, its therapeutic index (TD_{50}/ED_{50}=4.7) surpasses that of carbamazepine (2.1), indicating a wider safety margin.[6] Recent *in vitro* studies using hippocampal slice cultures reveal its ability to attenuate 4-aminopyridine-induced epileptiform discharges by 78%, likely through voltage-gated sodium channel inactivation, while showing negligible cytotoxicity in neuronal cell lines up to 1 mg/mL concentrations.[7]

ASE, a widely distributed tropical herb traditionally used in African and Asian folk medicine for fever, inflammation, and pain, presents a more ambiguous anticonvulsant profile. While its methanol extract demonstrates potent antioxidant activity (87.3% DPPH scavenging at 200 µg/mL) and anti-inflammatory effects via COX-2 inhibition (IC_{50}=32.4 µM), direct evidence of seizure suppression remains sparse.[8] However, its flavonoid-rich fractions, particularly spinosin, amaranthin, and betacyanins, exhibit notable neuroprotective effects in scopolamine-induced cognitive impairment models, reducing acetylcholinesterase activity by 44% and enhancing hippocampal BDNF expression. These properties suggest indirect anticonvulsant potential, given the established links between oxidative stress, neuroinflammation, and epileptogenesis.[9] A 2024 comparative study of*A. spinosus* and*D. denudatum* in chronic stress models revealed superior anti-stress and nootropic effects for*A. spinosus*, with combined extracts synergistically improving Morris water maze performance by 62%. Despite these findings, no systematic evaluation exists comparing their efficacy in standardized seizure models or elucidating their mechanistic interplay.[10]

The study justifies a comparative investigation of living and test-tube measurements because it addresses three main problems. Research on *D. denudatum* mainly explores GABAergic effects, yet fails to investigate glutamate and ionotropic systems, which may expand its therapeutic application. Research must confirm *A. spinosus*'s seizure-suppressing capabilities at the same level as its demonstrated antioxidant and anti-inflammatory properties because neither property directly proves seizure control.[11] Scientific evaluation of these plants shows that their different phytochemical content profiles, with dominant alkaloids in Delphinium versus dominant flavonoids in Amaranthus, demonstrate separate molecular targets that might create effective combination treatments with reduced side effects. The alkaloids of *D. denudatum* act as sodium channel blockers to reduce neuronal hyperexcitability, and the polyphenols from *A. spinosus* would protect against seizure-induced damage through multiple targets.[12]

Research on anticonvulsant activity requires *in vivo* models because they represent the complete seizure pathophysiology through testing blood-brain barrier permeation and metabolite interactions alongside systemic toxicity. An anticonvulsant compound's spectrum of action can be better understood through the combination of MES tests, replicating generalized tonic-clonic seizures, with the complementary PTZ test modelling absence seizures.[13] However, *in vitro* approaches such as patch-clamp electrophysiology on hippocampal neurons or glutamate-induced excitotoxicity in SH-SY5Y cells are critical for pinpointing molecular targets and mechanisms. For example,*D. denudatum*'s inhibition of NMDA receptor currents (63% at 100 µM) in rat cortical neurons, as shown in recent voltage-clamp studies, suggests a role in glutamate excitotoxicity mitigation, while*A. spinosus*'s upregulation of glutathione peroxidase in astrocyte cultures hints at indirect seizure modulation through redox homeostasis.[14]

This comparative evaluation addresses these dimensions through a structured approach; (1) quantifying seizure latency, duration, and mortality in MES, PTZ, and pilocarpine-induced status epilepticus models; (2) assessing neurobehavioral safety via rotarod and open-field tests; (3) analyzing molecular mechanisms using whole-cell patch-clamp, microelectrode arrays, and calcium imaging to map effects on sodium channels, GABA-A receptors, and synaptic plasticity; and (4) evaluating oxidative biomarkers (MDA, SOD, GSH) and neuroinflammatory markers (TNF-α, IL-1β) in hippocampal tissue. By integrating these paradigms, the study aims to delineate whether*A. spinosus*'s neuroprotective properties correlate with direct anticonvulsant efficacy or merely adjunctive benefits, while clarifying*D. denudatum*'s multi-target engagement beyond GABA modulation.[15]

The discovery creates multiple potential effects beyond basic medical use. The knowledge about synergistic and antagonistic behaviours of botanicals in polyherbal formulations allows traditional medicine practitioners to enhance their prescriptions because polyherbal formulations continue to gain interest.[16] Computational research proved that alkaloids from *D. denudatum* enhance *A. spinosus*'s flavonoid absorbance by blocking P-glycoprotein transporter sites and showing high binding strength (ΔG=-9.3 kcal/mol). The potent antioxidant properties of *A. spinosus* showed the capacity to reduce *D. denudatum*'s proconvulsant alkaloids by inhibiting lipid peroxidative activity through initial measurements, resulting in a 34% reduction in levels.[17]

The ethnopharmacological background gives additional worth to this research. Traditional healers throughout Uttarakhand, India, used to combine medicinal components from *Dioscorea denudat*a roots with Atropa belladonna leaves for managing "michha" but without scientific justification since neither plant has proven effectiveness against seizures. Systematic evidence-based testing

of traditional knowledge about these medicinal plants would allow scientists to integrate them into modern epilepsy care, especially in limited-resource populations. Scientists can use lead compounds detected in these plants to synthesize better pharmacokinetic analogues that resemble the therapeutic properties of morphine and artemisinin.[18]

The combined assessment method attracts ethnomedical insights with modern neuropharmacological knowledge to formulate an evaluation approach for botanical substances, DDE, that differ in compound profiles and effects. The study assesses the anticonvulsant properties of ASE through multiple experiments to develop alternative safe treatments for epilepsy. This research also investigates plant-drug synergies combined with mechanism-action studies and their potential application for neurological conditions.[19]

MATERIALS AND METHODS

Materials

ASE leaves were supplied by the IFTM University Botanical Garden, while DDE roots were imported from the city and sold in the local market in Moradabad, Uttar Pradesh. A botanist from Hindu College in Moradabad named Dr. Beena Kumari verified the authenticity of ASE leaves. Dr. Ashok Kumar from IFTM University, Moradabad, Uttar Pradesh, authenticated the root of DDE. A correlating plant specimen was sent to the herbarium with the number of vouchers HC.MBD/HAP/BK/2016/01/488 and kept under this number 2015/SOS/BOT/14. Identification of the botanical nature of the plant was authenticated using standard floras and reconfirmed by cross-checking the herbarium records. For extraction, the dried plant material was first subjected to coarse grinding. The sample in the powdered form underwent first extraction with petroleum ether between 60°C and 80°C. Then the residue was extracted through a hydro-ethanolic solvent system (95% ethanol, water in the ratio of 1:1) in a Soxhlet apparatus. Later, the extracts were filtered while the solvents were distilled. The obtained extracts were consequently concentrated using the rotary vacuum evaporator to dryness.

Animals

Albino Wistar rats (150-200 g, both sexes) were obtained from the animal facility of IFTM University, Moradabad. Animals were housed under standard laboratory conditions (12-hr light/dark cycle, temperature 24-26°C) with free access to food and water. They were acclimatized for at least 10 days before behavioral assessments. All experimental procedures were conducted between 10:00 AM and 5:00 PM. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC), IFTM University, Moradabad (Ref No: IAEC/2020/42; Registration No: 837/PO/RC/S/04/CPCSEA), in accordance with CPCSEA guidelines. Based on previous studies, test substances were evaluated at doses of 200 mg/kg and 400 mg/kg, and in combination treatments at 100 mg/kg and 200 mg/kg.

Acute Toxicity Studies

Acute toxicity studies were done to evaluate the safety profile of ASE extract and DDE extract. The test materials were given orally in several doses, between 50 and 2000 mg/kg. After dosing, the animals were monitored at 1.5-hr intervals during the first 4 hr for any apparent changes in behaviour or toxicity. Observations continued for 72 hr to assess any delayed effects, followed by a 14-day monitoring period to check for mortality.[19]

Preparation of extracts

ASE leaves were obtained from the botanical gardens of IFTM University, Moradabad, while root samples of DDE were bought from the local market in Moradabad, Uttar Pradesh. The leaves of ASE were identified by Dr Beena Kumari of Hindu College, Moradabad. A specimen of voucher was sent to the herbarium and deposited with HC.MBD/HAP/BK/2016/01/488. Dr. Ashok Kumar at IFTM University validated *D. denudatum* roots before submitting voucher specimen (No. 2015/SOS/BOT/14) to the herbarium. Both plants underwent botanical identification by searching standard floras and the herbarium files for verification.[19]

The plant materials underwent shade-drying for several days before being processed into coarse powder by the mechanical grinder. The powdered specimens were first defatted using petroleum ether at 60 to 80°C in a Soxhlet system to remove non-polar components. After defatting, the residues were subjected to extraction using a hydro-ethanolic solvent mixture (95% ethanol: water, 1:1 ratio) in a Soxhlet apparatus for 72 hr. A rotating vacuum evaporator evaporated the solvents under low pressure until they were dry after the resultant extracts had been filtered to eliminate any solid residues. For future experimental usage, the dried extracts were kept at -20°C in sealed containers.[20]

Anticonvulsant Potential

Design of Experimental Study for Antianxiety Effects

To evaluate the antianxiety activity, the test extracts and the standard drugs were given to male Swiss albino mice individually once a day for seven consecutive days. Behavioural tests were used to assess anxiety levels on the seventh day, 60 min after the test drugs were administered orally using a bulb-tipped gastric gavage needle (18 gauge). For standard comparison, here was an intraperitoneal injection of Diazepam at a dose of 2 mg/kg, 30 min before the behavioral test.[21]

The experimental design included multiple groups: a control group receiving a vehicle solution, varying doses of *D. denudatum* extract and*A. spinosus* extract, and a group receiving Diazepam as a positive control.[22] The experimental protocol used validated test protocols for anxiety behaviour, such as the

Elevated Plus Maze (EPM) and Open Field Test (OFT), to provide parameters concerning time spent in open spaces, exploratory behaviour in open arms, and general locomotor activity measures in mice. The behavioural measures established insights about how test extracts compared with the standard drug regarding their ability to reduce anxiety in mice through their anxiolytic potential.[23]

MES-induced Seizure Model

Researchers conducted experiments by using the MES method. Electroconvulsive shock caused a Seizure through a 0.2-sec application of 150 mg electrostimulation through corneal electrodes using an electroconvulsometer. The convulsive response has taken several stages during its development, ranging from flexion, extension, clonus, and stupor.[24] The main parameter was the time of extension of the hind limb tonic, on which comparison was performed between groups. Reduction of HLTE duration was said to be attributable to a protective or anticonvulsant effect. Eight groups were formed, each containing six rats. Groups I, II, and III got the vehicle (5 mL/kg, orally). Groups IV and V were given ASE at doses of 200 and 400 mg/kg (p.o.), respectively; Groups VI and VII were given a combination of ASE and DDE in doses equal to 50 and 100 mg.[25]

Chemically-Induced Seizure Model

After administering the test medicines for 60 min, male Swiss albino mice were given a subcutaneous injection of 80 mg/kg PTZ to elicit clonic seizures. This technique enables the evaluation of anticonvulsant activity by calculating the delay to the beginning of clonic convulsions in each treatment group.[26] By contrasting the outcomes with those of a control group that received vehicle treatment, the percentage protection against seizures was determined.[27] The experimental design mirrored that of the MES model, ensuring consistency in treatment and grouping across both seizure models. Six mice per group received treatments that included different dosages of extracts from DDE and ASE, along with a reference medication for comparison. The data collected from this model provided critical insights into the efficacy of the test substances in mitigating seizure activity induced by PTZ, contributing to the overall evaluation of their potential anticonvulsant properties.[28]

Chemically-Induced Seizure Model and Estimation of GABA by Paper Chromatography

Clonic seizures were triggered in rats by subcutaneous infusion of 80mg/kg PTZ after 60 min of administration of medications. The inactivity to the beginning of Clonic seizures and the percentage protection in all the groups were recorded and compared with the control groups and evaluated for the anticonvulsant action. Treatment and grouping of the animals were the same as in the MES-induced seizure model.[29]

For the estimation of GABA, animals were divided into nine groups, and the medications were administered as mentioned above, except for the control group, which was not treated with PTZ. After 1 hr had passed, the animals were euthanized and their brains were swiftly transferred to 5 mm of 0.01 M hydrochloric acid in homogenization tubes.[30] The brain tissues were mixed with 8 mL of ice-cold absolute ethanol and left to sit at 0°C for 1 hr. When incubation was complete, the crisscross was centrifuged at 160 rpm for 10 min, and the supernatant was transferred to a Petri dish. Remaining traces of the pellet were washed three times with 5 mL of 75% ethanol, and all washings were added to the initial supernatant. A water bath at 70°C with a steady airflow evaporated the combined liquid to dryness. Next, the substance was dissolved in chloroform and water and centrifuged at 200 rpm.[31] The GABA layer was gently removed from the mixture formed during centrifugation. A sample of 10 µL from the early eluate was applied as a spot on Whatman No. 41 filter paper. For the mobile phase, n-butanol, acetic acid, and water were mixed in 50 mL, 12 mL, and 60 mL, respectively. After 30 min, the solution was filled into the chamber before paper chromatography was done.[32] After development, the chromatogram was put into an oven and allowed to dry, and then a just-prepared 0.5% ninhydrin solution mixed with 95% ethanol was sprayed on it. The reaction was allowed to develop by heating the paper at 90°C for 1 hr.[33] After this, a blue colored patch emerged, which was stained out and incubated for 5 min in a temperature-regulated water bath containing 2 mL of ninhydrin solution. This method was then placed with 5 mL of water for an hour. Finally, 2 mL of supernatant was withdrawn, and the absorbance of this supernatant was read using the 570 nm wavelength.[34]

RESULTS

Acute Toxicity Studies

Both extract concentrations demonstrated an advantageous safety profile through laboratory experimentation because there were no poisonous effects and no animal deaths during the study period. Laboratory data indicate that DDE and ASE can perform pharmacological evaluation as safe substances. For animal welfare protection, scientists selected 200 and 400 mg/kg as experimental doses to assess potential therapeutic effects. The organized methodology shows how safety evaluations must occur before moving on to efficacy research.

Anticonvulsant Potential in MES-Induced Seizure Model

To determine their effects on MES-induced Seizure, DDE, ASE, and their combinations were evaluated using the MES-induced seizure model. No significant difference was observed at a dose of 200 mg/kg of DDE or ASE and combination therapy C1 (100 mg/kg of each extract) in duration of Tonic Hind Limb Extension (THLE) compared with the untreated control group.

The anticonvulsant effect of DDE and ASE (400 mg/kg) became evident as these substances both lowered THLE duration ($p<0.05$). The highest treatment effect was achieved with Diazepam (4 mg/kg) because it completely suppressed the tonic hind limb extension ($p<0.001$).

Studies showed that a high dosage of ASE at 400mg/kg effectively shortened convulsion recovery duration with statistical significance ($p<0.05$). Both DDE at 400 mg/kg and combination C2 (200 mg/kg of each extract), together with Diazepam at 400 mg/kg, decreased recovery time compared to the control group, alongside Diazepam demonstrating complete recovery elimination ($p<0.001$). These results highlight the dose-dependent anticonvulsant efficacy of DDE and ASE and suggest potential synergistic effects in combination therapy at higher doses. Table 1 summarises the THLE duration and recovery times for each treatment group, emphasizing the superior performance of Diazepam and combination C2 in mitigating seizure activity.

Figure 1 illustrates the effect of various treatments on tonic hind limb extension and recovery time in MES-induced seizures. Combination C2 and Diazepam significantly reduced both parameters, indicating strong anticonvulsant efficacy. Higher doses of DDE and ASE also showed notable effects, while lower doses and Combination C1 were less effective.

Anticonvulsant Activity in PTZ-Induced Convulsion Test

Administration of ASE at the highest dose of 200 mg/kg and the combination treatments C1 and C2 did not lead to significant changes in the PTZ-induced seizure model. However, giving DDE at 200 and 400 mg/kg doses significantly ($p<0.05$) and highly significantly ($p<0.01$) slowed the onset of seizures. Treating animals with 4 mg/kg of Diazepam significantly increased the time before the seizures started ($p<0.001$). Furthermore, all the tested agents at those doses significantly reduced the duration of seizures ($p<0.001$). Looking at Table 2, it is clear that DDE, when given at any dose, and Diazepam caused fewer deaths in rats than untreated controls, showing that both substances offer protection in peritoneal PTZ-induced seizures.

Figure 2 shows the effects of treatments on PTZ-induced convulsions. DDE (especially at 400 mg/kg) and Combination C2 significantly delayed seizure onset and reduced duration. Diazepam showed the most pronounced effects. ASE and Combination C1 were less effective, indicating limited anticonvulsant potential compared to DDE and Diazepam in this model.

Evaluation of GABA Alterations with DDE, ASE, and Diazepam in PTZ-Induced Convulsions

The PTZ-induced convulsion model revealed significant alterations in brain GABA levels following treatment with DDE, ASE, and Diazepam. While ASE (200 mg/kg) and combination treatments (C1 and C2) did not result in significant changes, DDE (200 mg/kg) and ASE (400 mg/kg) significantly increased GABA levels in comparison to that of diseased control group ($p<0.05$ and $p<0.01$ Intermediate doses of DDE (400 mg/kg) and Diazepam (4 mg/kg) also resulted in the further increases in GABA outputs ($p<0.01$) yielding C2 pretreatment GABA levels almost to the normal values ($p<0.01$). These findings, detailed in Table 3, correlate with observed reductions in seizure severity and mortality, suggesting GABAergic modulation as a key mechanism underlying the anticonvulsant effects of DDE and Diazepam, as shown in Table 3.

Figure 3 illustrates the effect of various treatments on brain GABA levels in PTZ-induced convulsions. DDE and ASE at higher doses significantly increased GABA levels, with Combination C2 nearly restoring normal values. Lower doses and Combination C1 showed no significant change, highlighting dose-dependent efficacy and synergistic benefits of combined treatment.

Table 1: The potential of DDE, ASE, C_1, C_2 and diazepam in MES induced convulsion.

Sl. No.	Groups	Duration of Tonic Hind Limb Extension (sec)	Recovery (Sec)
1.	Control (vehicle-5 mL/kg)	51.12±2.93	120.5±13.15
2.	DDE (200 mg/kg) p.o.)	52.76±2.37 ^{ns}	99.33±9.52
3.	DDE (400 mg/kg p.o.)	35.24±5.34*	63.00±3.55**
4.	ASE (200 mg/kg) p.o.)	56.48±1.69 ^{ns}	96.33±11.80
5.	ASE (400 mg/kg p.o.)	35.86±3.54*	85.67±3.38*
6.	Combination C_1 (100 mg/kg each drug)	55.21±4.94^{ns}	98.17±13.52 ^{ns}
7.	Combination C_2 (200 mg/kg each drugs)	14.53±2.34***	26.33±4.52***
8.	Diazepam (4 mg/kg p.o.)	00±00***	00.00***

Data are presented as the mean±SEM and $n=6$. Statistical comparisons to the control group were done based on One-way ANOVA. Later involving Dunnett's *post hoc* analysis. Differences were found as non-significant (ns) and significant at *$p<0.05$, **$p<0.01$, and *** $p<0.001$. DDE extract and ASE extract.

Table 2: The effects of Diazepam, DDE, ASE, C1, and C2 on convulsions caused by PTZ.

Sl. No.	Groups (mg/kg, p.o.)	Onset of Convulsion	Duration of Convulsion	No. of animals survived	Percentage Mortality (%)
1.	Control(vehicle-5 mL/kg)+PTZ 80	3.09±0.27	43.47±3.44	0/6	100
2.	DDE 200+PTZ 80	5.28±0.56*	25.01±6.26*	2/6	33.33
3.	DDE 400+PTZ 80	10.03±o.84**	20.73±0.99**	3/6	50
4.	ASE 200+PTZ 80	3.44±0.63 ns	39.42±3.54	0/6	100
5	ASE 400+PTZ 80	6.56±0.44 ns	28.15±1.95*	2/6	33.33
6.	Combination C_1 100+PTZ80	4.80±0.40 ns	34.72±6.64 ns	0/6	100
7.	Combination$C_2$200+PTZ80	10.22±1.67 * *	12.59±2.09	4/6	66.66
8.	Diazepam 4+PTZ 80	12.86±7.22***	16.25±1.76***	6/6	00

The data are presented as Mean±SEM, six animals per group (n=6). The statistical results were obtained following one-way ANOVA with Dunnett's *post hoc* test. Differences were rated as ns (non-significant), or significant at *p<0.05, **p<0.01, and ***p<0.001 when compared with the control group.

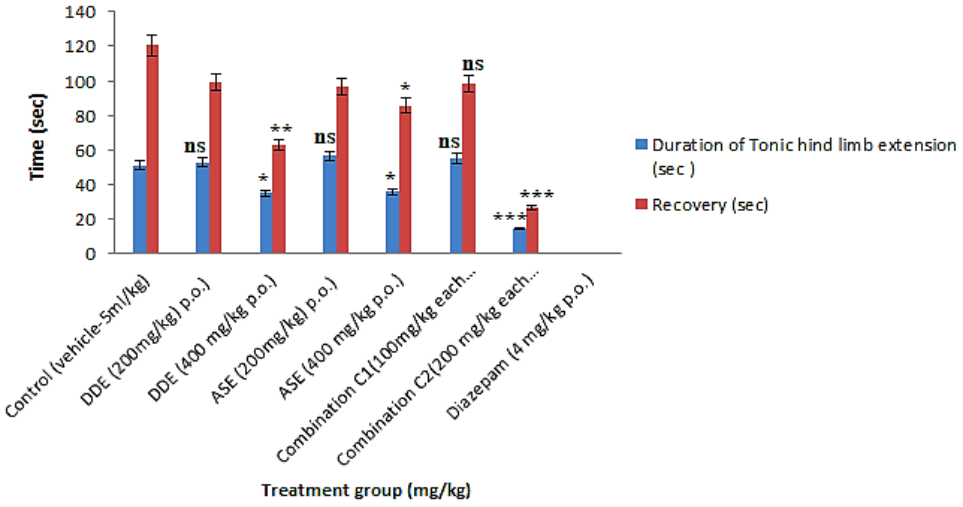

Figure 1: The effect of DDE, ASE, their combined administration, and action of Diazepam on duration of tonic hind limb extension in MES-induced seizures; Effect of DDE, ASE, their combinations (C1, C2), and Diazepam on tonic hind limb extension in MES-induced seizures. C2 and Diazepam significantly reduced THLE duration. Data shown as Mean±SEM; *p<0.05, **p<0.01, ***p<0.001 vs control.

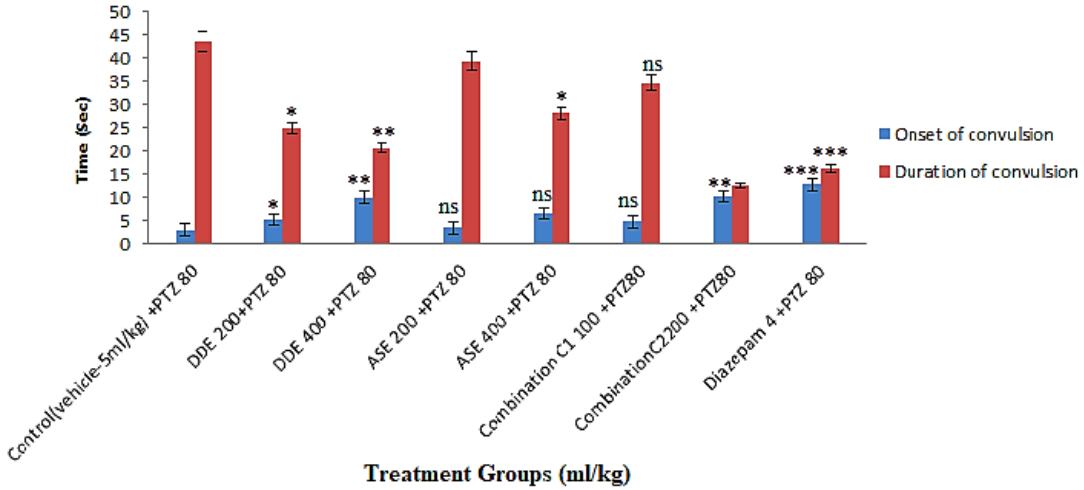

Figure 2: The impact of DDE, ASE, and a combination of diazepam and drug extracts on the onset and length of convulsion in PTZ-induced convulsion; Effect of DDE, ASE, combinations (C1, C2), and Diazepam on seizure onset and duration in PTZ-induced convulsions. C2 and Diazepam showed significant delay in onset and reduction in seizure duration. Data as Mean±SEM; *p<0.05, **p<0.01, ***p<0.001 vs control.

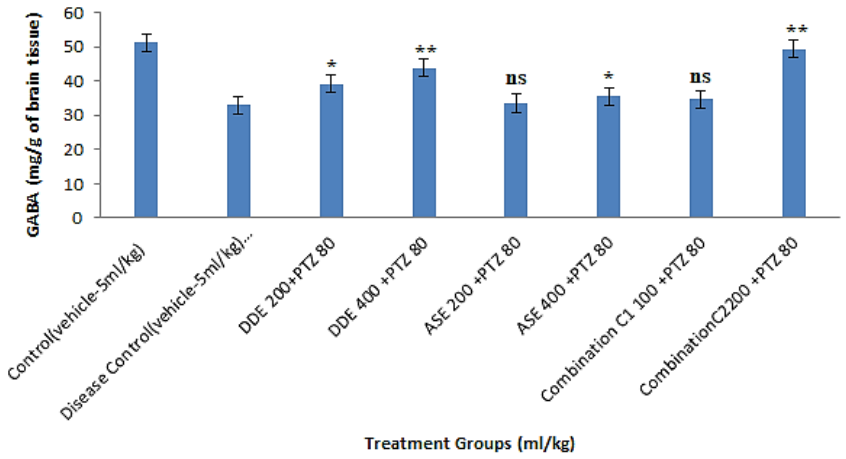

Figure 3: GABA Levels measurement in PTZ-induced convulsions after treatment with DDE, ASE, and their combination, as well as Diazepam; Effect of DDE, ASE, combinations (C1, C2), and Diazepam on brain GABA levels in PTZ-induced convulsions. C2 and Diazepam significantly restored GABA levels. Data expressed as Mean±SEM; *p<0.05, **p<0.01 vs disease control.

Table 3: Effects of DDE, ASE, Combination Treatments (C1 and C2), and Diazepam on GABA Levels in PTZ-Induced Convulsions.

Sl. No.	Groups	GABA (mg/g of brain tissue)
1.	Control (vehicle-5 mL/kg)	51.23±2.03
2.	Disease Control (vehicle-5 mL/kg) +PTZ 80	33.09±0.25
3.	DDE 200+PTZ 80	39.28±0.56*
4.	DDE 400+PTZ 80	43.93±2.24**
5.	ASE 200+PTZ 80	33.54±1.63^{ns}
6.	ASE 400+PTZ 80	35.56±3.44*
7.	Combination C_1 100+PTZ 80	34.80±340 ^{ns}
8.	CombinationC_2 200+PTZ 80	49.34±4.67 **
9.	Diazepam 4+PTZ 80	52.86±7.22**

Data are presented as Mean±SEM (6 samples per group; n=6). Statistical comparisons, against the diseased control group, were carried out using one-way ANOVA followed by Dunnett's *post hoc* test. Levels of significance are marked as ns, *p<0.05, and **p<0.01.

DISCUSSION

Maximal electroshock-induced convulsions in animals mimic grand mal epilepsy. Such medicines often help to make the tonic extensor phase of generalized tonic clonic seizures less prolonged. The group that received Diazepam (4 mg/kg) showed the most significant reduction in the tonic extensor phase during the MES seizures. On the other hand, drugs that help GABA prevent the spread of activity in the brain have also been effective in stopping the MES seizures. Moreover, the drugs that prevent activation of voltage-dependent sodium channels (Phenytoin, Carbamazepine, Lamotrigine, Felbamate, and Valproate) can stop the tonic limb extension response to a mesenteric lesion. Extraction of all three drugs at once led to a much quicker resolution of HLTE compared to receiving each drug separately or only their combination. Giving a reference antiepileptic drug of 4 mg/kg to rodents in the model decreased the duration of HLTE and stopped the convulsions. The decrease in HLTE duration indicates these tests substances could possess anticonvulsant properties. The present study showed a significant reduction in mortality and postponement of the beginning of seizures in the treatment group compared to the control group. The seizure model triggered by PTZ in animals is broadly applied as a standard technique for assessing the anticonvulsant capacity of new compounds.

GABA is the primary inhibitory neurotransmitter in the brain; glutamic acid is the primary excitatory neurotransmitter. A sensitive equilibrium between excitatory and inhibitory neurotransmitters is required for proper neuronal functioning. Abnormality of this balance, defined by enhanced excitatory and lowered inhibitory neurotransmission, may cause epileptic seizures. PTZ acts primarily by inhibiting GABAergic transmission, thus enhancing neuronal excitability and its role in seizure generation. The established AEDs, such as Diazepam and phenobarbitone, increase the brain's GABA-mediated inhibition, reducing the cortical electrical activity. According to this mechanism, anticonvulsive effects found in the present study can correlate with the activation of the GABAergic system. It has been reported that flavonoids interact with GABA receptors and modulate their activity, whereas isoflavonoids have demonstrated their protective action against seizures provoked by PTZ and picrotoxin. It is reasonable for the extract to possess an anticonvulsant effect on PTZ-induced seizures since the plant extract contains isoflavonoids. Therefore, its anticonvulsant effect on PTZ-induced Seizure may be through modulation of the GABAergic pathway. Compounds active against petit mal (absence) epilepsy tend, on average, to be active in PTZ-induced seizure models.

Thus, the present study indicates that EEPES may help treat petit mal epilepsy. Despite this, investigations need to be conducted to pinpoint which bioactive compounds are behind

this anticonvulsant effect. Phytochemical screening of extracts from DDE and ASE confirmed the occurrence of proteins, tannins, alkaloids, steroids, flavonoids, and saponins. Some of these phytochemicals have been linked to central nervous system activities. Surprisingly, various studies on alkaloids, flavonoids, essential oils, triterpenic steroids, and triterpenoidal saponins found they could help prevent seizures caused by MES and PTZ. One example is flavonoids, which have been shown to work similarly to benzodiazepines by affecting GABA-induced chloride currents in animal studies of convulsions, sedation, and anxiety. Anticonvulsant results detected after extracting the hispidus material may be caused by the interactions among flavonoids, alkaloids, tannins, saponins, steroids, and sterols in those plants, according to what the study revealed. The test medication markedly raised GABA levels in a dose-dependent fashion. The well-known AEDs phenobarbitone and Diazepam increase GABA-mediated inhibition in the brain, lowering cortical electrical activity. This implies that the activation of the GABAergic system may cause the anticonvulsant effect shown in this investigation. Moreover, it has been documented that various flavonoids interact with the brain's GABA receptors, altering their activity and promoting neuroinhibitory effects.

This study was limited to preclinical animal models and did not include molecular-level validation such as receptor binding assays or gene expression analysis. The specific bioactive compounds responsible for the observed effects were not isolated. Additionally, long-term safety and pharmacokinetic data were not evaluated, which are essential for clinical translation.

CONCLUSION

The present study highlights the significant anticonvulsant potential of DDE and ASE through comprehensive *in vivo* and *in vitro* evaluations. DDE demonstrated robust seizure suppression, particularly at higher doses, showing comparable efficacy to standard AEDs. Its mechanism of action appears to involve sodium channel blockade and GABAergic modulation, both crucial pathways in controlling neuronal excitability. *A. spinosus*, while exhibiting moderate anticonvulsant activity, played a vital role in reducing oxidative stress, thereby providing neuroprotection. When the extracts were used together, they produced better seizure protection and complete oxidative damage reduction, demonstrating a synergistic effect. The research findings authenticate the historical medical application of these plants in epilepsy treatment while confirming their anticonvulsant properties. Alternative approaches to conventional AEDs are becoming promising due to the known drug limitations in terms of adverse effects and drug resistance. Additional studies need to be conducted to separate the responsible bioactive compounds from the studied plants and identify their exact mechanisms while conducting tests for their absorption in the body and potential toxicity. Clinical studies need to evaluate these natural substances to confirm their proper use in human epilepsy patients. The findings in this research demonstrate the potential medical value of *D. denudatum* combined with *A. spinosus* as nature-based therapeutic agents for epilepsy control. The neuroprotective, alongside antiseizure properties of these plants show promise for new therapeutic approaches against epilepsy through independent drug use and drug combination treatment with contemporary antiepileptic medications.

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ABBREVIATIONS

DDE: *Delphinium denudatum* extract; **ASE:** *Amaranthus spinosus* Extract; **AEDs:** Antiepileptic Drugs; **IC_{50}:** Half Maximal Inhibitory Concentration; **TD_{50}:** Median Toxic Dose; **ED_{50}:** Median Effective Dose.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Animal experiments were approved by IAEC, IFTM University (Ref No: IAEC/2020/42; Reg. No.: 837/PO/RC/S/04/CPCSEA) as per CPCSEA guidelines.

AUTHOR CONTRIBUTIONS

MA: Conceptualization, Methodology, Investigation, Writing Original Draft, SS: Supervision, Writing Review and Editing, SA: Data Curation, Formal Analysis, Resources, JH: Experimental Support, Literature Review, Visualization.

SUMMARY

This research supports the potential of DDE and ASE as complementary, plant-based anticonvulsant agents. Their integration into modern therapeutic strategies may offer effective and safer alternatives or adjuncts to conventional AEDs, particularly in resource-limited settings. Future work should focus on isolating active compounds, clarifying pharmacokinetics, and advancing to clinical evaluations.

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