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Anticonvulsant and antioxidant activity of aqueous leaves extract of *Desmodium triflorum* in mice against pentylentetrazole and maximal electroshock induced convulsion

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Abstract: The present investigation was aimed to study an anticonvulsant activity of aqueous extract of *Desmodium triflorum* (L.) DC., Fabaceae, in mice. Animal models of epilepsy namely the pentylentetrazole, and maximal electroshock induced convulsion were used to evaluate the anticonvulsant effects of the extracts. The biochemical estimation was done by measuring the lipid peroxidation and reduced glutathione. In the pentylentetrazole induced convulsion, aqueous extract of *D. triflorum* 800 mg/kg significant delayed the onset of convulsion, reduced the duration of convulsion ($p < 0.05$) and reduced mortality. The aqueous extract of *D. triflorum* 800 mg/kg dose reduced hind limb tonic extension phase of maximal electroshock induced convulsion in mice ($p < 0.05$). The pretreated aqueous extract of *D. triflorum* showed significant inhibition of lipid peroxidation and increases the reduced glutathione level in mice brain tissue ($p < 0.001$). The results revealed that *D. triflorum* possesses a significant dose dependent anticonvulsant activity.

Introduction

Epilepsy is a chronic brain disorder characterized by recurrent derangement of the nervous system due to sudden excessive disorderly discharge from the cerebral neurons (Maiha et al., 2009). It is the second most common neurological disorder with an annual incidence of 50 cases/100000 per year. Overall it accounts for 1% of the world's burden of diseases, and the prevalence rate is reported at 2% (Vyawahare et al., 2007). Seizures are controlled in nearly 70% of patients with epilepsy, mostly through drugs effect on membrane ion channels or on gamma amino butyric acidergic (GABA) or glutamatergic transmission (Marjan et al., 2009). World Health Organization (WHO) estimated that approximately 80% people with epilepsy live in developing countries and most of them do not get adequate medical treatment (Reddy, 2005). All the currently available antiepileptic drugs are synthetic molecules (Hema et al., 2009). In many patients, the presently available antiepileptic drugs (AED) such as phenobarbital, phenytoin, benzodiazepines, sodium valproate, carbamazepine, ethosuximide, trimethadione etc., are unable to control seizures efficiently. Furthermore, the dose-related neurotoxicity and other side effects associated with established AED limit their clinical use. The newer AED like oxcarbazepine, vigabatrin,

lamotrigine, gabapentin, felbamate etc., represent a real progress in the treatment of no responders or refractory patients. However, the problem of adverse effects has also not been circumvented completely and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy (Ezekiel et al., 2010). Hence, search should continue to develop newer, more effective, and safer neuroprotective agents for treatment of epilepsy. Medicinal plants used in traditional medicine for the treatment of epilepsy have been scientifically shown to possess promising anticonvulsant activities in animal models for screening for anticonvulsant activity (Wannang et al., 2008).

Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, the last few years have seen a major increase in their use in the developed world. Global estimates indicate that 80% of about 4 billion population cannot afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional medicines which are mainly derived from plant material (Joy et al., 2001). Considering the great reliance on traditional medicinal plants for treatment of diseases and the potential for drug discovery; it becomes relevant

to search for potent, effective and relatively safe plant medicines.

Of late, *Desmodium triflorum* (L.) DC., Fabaceae, is a perennial herb belonging to the family Papilionaceae (Kirtikar & Basu, 1980). The plant is available in all tropical countries. It contains hypaphorine (major alkaloid), *N,N*-dimethyltryptophan betain and choline (Ghosal et al., 1971). *D. triflorum* leaves contains total alkaloid, 0.01-0.015% and rare diholosylflavone, 2-*O*-glucosylvitexin had been isolated from *D. triflorum* (Adinarayana & Syamsundar, 1982). It also contains ursolic acid, vitexin, genistin, fucosterol (Yoganarasimhan, 1996). In traditional medicinal system, different parts of the plant have been mentioned to be useful in a variety of diseases. The leaves are used in diarrhoea, convulsions, anti-spasmodic, sympathomimetic, central nervous system stimulation, curare-mimetic activity and diuretic and as a galactagogue (Yoganarasimhan, 1996). The fresh leaves of the plant are applied to wounds and abscesses that are usually difficult to heal. The paste is sometimes applied to sores and itch. The fresh juice of the plant is often given to the children for coughs and asthma. The traditional use of the plant also recommends for use in dysentery and as a laxative (Adinarayana & Syamsundar, 1982), high fever (Samvatsar, 2004) and cure bone-fracture (Prusti & Behera, 2007). Different extracts of *D. triflorum* exhibit analgesic and anti-inflammatory activities (Kawshik et al., 2005) and also possesses antioxidative and antiproliferative activities. (Mao et al., 2007; Lai et al., 2010). The antinociceptive activity of cold water extract of *D. triflorum* in rats (Daya et al., 2011) and antioxidant activities of phenolic components from various plants of *Desmodium* species were studied (Tsai et al., 2011). Recently Evaluation of anticonvulsant activity of ethanolic leaves extract of *Desmodium triflorum* in mice was studied and it possesses very good anticonvulsant activity (Gowda et al., 2012) however, only limited data are available concerning the anticonvulsant activity of this plant species hence the present work was undertaken to evaluate the anticonvulsant activity of an aqueous extract of *D. triflorum* leaves.

Material and Methods

Plant material

Desmodium triflorum (L.) DC., Fabaceae, was collected from Gandhi Krushi Vignana Kendra (GKVK) Bangalore. The plant was identified and authenticated by Dr. Siddamallayya, National Ayurvedic Dietetics Research Institute Bangalore. The voucher specimen is preserved in our laboratory for future reference (Voucher No. Drug Authentication/SMPU/NADRI/BNG/2010-11/286).

Preparation of extract

The leaves of the plant species collected were dried under shade for a period of four weeks. The dried plant material was milled to a fine powder using the commercial laboratory blender. Dried powder (300 g) was extracted in a Soxhlet extractor with ethanol for about 8-9 h at 45 °C. Extract was collected and dried using rotary flash evaporator at 40-45 °C and crude residue was collected. The yield was calculated as 30 g. The extract was stored in well closed glass container at 5 °C in refrigerator for further study.

Preliminary phytochemical analysis

The extracts obtained were subjected to various chemical tests to detect the chemical constituents present in them (Trease & Evans, 1983; Khandelwal, 1996).

Animals

Healthy Swiss albino mice of either sex (18-20 g) were procured from the Central Animal Facilities of the Drug Testing Laboratory (DTL), Bangalore. Animals were housed at our Institute's animal house facilities until they gained significant weight (25±5 g) suitable for the present investigation. The estrous cycle in female mice was observed normal. They were housed in hygienic cages (polypropylene cage) and maintained under standard laboratory conditions for one week before the experiments started and were kept in groups of six per cage at controlled temperature (22±2 °C) with 12 h light/dark cycle and humidity (50%). They received standard diet and water ad libitum. The animals were maintained in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals) guidelines for the care and use of laboratory animals. Approval for the experiment was obtained from the Institutional Animal Ethics Committee (IAEC), St. John's Pharmacy College, Vijayanagar, Bangalore, letter No. IJAHS/ IAEC/2010-04.

Acute toxicity study

Acute toxicity study for the aqueous and aqueous extract of *D. triflorum* was done according to the OECD guidelines No: 423 and low and high dose was selected for treatment. The aqueous extract (70.0%) of *D. triflorum* was administered orally in the escalating dosages, up to 2000 mg/kg to different groups of rats (n=6, in each). The animals were observed for behavioral and physiological variations initially continuously for 4 h, followed by 4th hourly for 12 h and there after once daily for fourteen days. If toxic signs or lethality is not observed, then 1/5th, 1/10th and 1/20th part of the limit test dose were considered as test

doses for the present investigation.

Anticonvulsant screening method

Pentylenetetrazole (PTZ) induced convulsion

Animals were divided into IV groups, (n=6 mice of either sex in one group). Group I received respective vehicles (2% of Tween 80); group II was allotted for standard drug (diazepam 5 mg/kg) and group III, and IV (400 and 800 mg/kg body weight) received AEDT at different dose levels. All treatment and standard groups were statistically compared with vehicle groups. Vehicles, extracts are administered by oral route and standard drugs were administered by intra-peritoneal (*i.p.*) route. Mice were administered extracts for seven days and on the experimental day, PTZ 65 mg/kg was injected intra-peritoneally to mice 45 min after vehicle or extracts and 30 min after the standard drug. Immediately after PTZ administration mice were observed for (1) onset of convulsions (elapsed time from PTZ injection until convulsion occurred), (2) duration of convulsion (number of mice showing convulsions) and (3) mortality for the duration of 30 min (Swinyard et al., 1952).

Maximum electro shock (MES) induced convulsions

The electroshock was applied via ear-clip electrodes separately to each mouse. The stimulus duration was 0.2 s and the current frequency 45 mA (60 Hz). Six mice of either sex in one group with a weight of 25±5 g, mice were administered extracts for seven days and on the experimental day, test was started 60 min after administration of extracts and 30 min after standard drug (phenytoin 25 mg/kg *i.p.*). The animals were observed for the occurrence of tonic hind limb extension and mortality for duration of 15 min (Swinyard et al., 1952). The AEDT was administered to Group III and IV (400 and 800 mg/kg body weight) where as group I and II received 2% of Tween 80 and Phenytoin 25 mg/kg *i.p.*, respectively.

Biochemical estimation

Tissue preparation

Three groups were used (n=6). Group I animals (control) were administered 2% tween 80 (2.0 mL/kg, *p.o.*), Groups II and III animals were administered 400 and 800 mg/kg of the AEDT orally. One hour after administration of the extract, PTZ (65 mg/kg) was injected *i.p.* to all the animals in Groups. On observing onset of convulsions, duration of seizure following the administration of PTZ, the animals (including control group) were sacrificed by decapitation and brain was removed, homogenized in

0.9% NaCl by using Remi motor RQT-1.2.7A.

Lipid peroxidation in brain

Two milliliter of suspension medium was taken from 10% of tissue homogenate. To this, 2 mL of 30% of trichloroacetic acid was added, followed by 2 mL of 0.8% thiobarbituric acid (TBA) reagent. The tubes were covered with aluminum foil and kept in shaking water bath for half an hour at 80 °C after half an hour; the tubes were taken out and kept in ice cold water for half an hour. There were then centrifuged at 3000 x g for 15 min. The absorbance of the supernatant was read at 535 nm at room temperature against appropriate blank. Blank consist of 2 mL distilled water, 2 mL of 30% TCA and 2 mL of 0.8% TBA (Ohkawa et al., 1979). The content of malonaldehyde (MDA), expressed as n moles formed per milligram of protein in the tissue, was calculated using the formula:

$$\text{Concentration} = A \times (V/E) \times P$$

Where, A is the volume of solution, E is extinction coefficient ($1.56 \times 10^5 \text{ m}^{-1} \text{ cm}^{-1}$) and P is the protein content of tissue calculated as milligram of protein per gram of tissue.

Brain glutathione

To 2 mL of 10% of homogenate, which was prepared in sodium chloride solution, 2.5 mL of 0.02M EDTA was added and shaken vigorously. To 2 mL of this mixture 4 mL of cold distilled water and 1 mL of 50% trichloroacetic acid were added and shaken for 10 min. Thereafter, the content were centrifuged at 3000 x g for 15 min following centrifugation, 2 mL of the supernatant was mixed with 0.4M tris buffer (pH 8.9). The whole solution was mixed well and 0.1 mL of 0.01M DTNB was added, the absorbance was read within 5 min of addition of DTNB at 412 nm against reagent blank with no homogenate. For blank reading, the homogenate was substituted by 2 mL of distilled water (Sedlak & Lindsay, 1968). The amount of glutathione in tissue was expressed as $\mu\text{mol/g}$ of tissue.

$\mu\text{mol/mg wet tissue: } [A/13600] \times \text{dilution factor} \times 1000.$

Statistical analysis

The data obtained by the various parameters was statistically evaluated by one way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test by Graph Pad Prism software (GraphPad software Inc., Version 5.0.0). The mean values±SEM were calculated for each parameter. Level of significance was kept at $p < 0.05$.

Results

Preliminary phytochemical screening

Phytochemical analysis of the aqueous extract of *D. triflorum* (AEDT) revealed the presence of alkaloids, proteins, flavonoids, phytosterol, saponin and tannins. However, the tests show that they do not contain carbohydrate, glycoside and fixed oil and fats.

Acute oral toxicity

Acute oral toxicity studies revealed the non-toxic nature of the AEDT. Aqueous extract and aqueous extracts of *D. triflorum* did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose after fourteen days of study. This indicates that the extracts were found to be safe up to the dose levels studied. Since, all the animals survived at a dose of 2000 mg/kg body weight, the LD50 of the extract will be >2000 mg/kg body weight. No major behavioral changes were observed during the period of study.

Anti-convulsant activity

Effect of extracts on pentylenetetrazole induced convulsion

Pentylenetetrazole (65 mg/kg, *i.p.*) produced convulsion in all the animals used. All extracts groups compared with the control group. Mice pretreated with AEDT at the dose of 400 mg/kg *p.o.* not showed significant delay the onset of convulsion and duration of pentylenetetrazole induced seizures in mice and reduced mortality to 33.33%. Similarly a dose of 800 mg/kg *p.o.* of AEDT significantly delayed the onset of convulsion ($p<0.05$), reduced the duration of convulsion ($p<0.05$) and not found any mortality. The standard anti-epileptic drugs, Diazepam (5 mg/kg) blocked the clonic convulsions and mortality in mice against pentylenetetrazole induced convulsion (Table 1).

Effect of extracts on MES-induced convulsion

The aqueous extract of *D. triflorum* caused

significant decrease in the duration of hind limb tonic extension (HLTE) induced by maximal electroshock. The AEDT group was compared with the control group. AEDT dose of 400 mg/kg *p.o.* not showed significant reduction HLTE and reduced the mortality to 33.33%. AEDT dose of 800 mg/kg *p.o.* significantly reduced the duration of HLTE ($p<0.05$) and no mortality was found against MES induced convulsion. Phenytoin (25 mg/kg *i.p.*) significantly reduced the duration of MES-induced HLTE ($p<0.01$) and completely prevented the various phases of convulsion induced by MES (Table 2).

Effect on brain lipid peroxidation

The effect of extract was showed significant inhibition of lipid peroxidation in mice brain tissue as compared to control group. The aqueous extract of *D. triflorum* 400 and 800 mg/kg *p.o.* dose showed significant ($p<0.001$) inhibition in malondialdehyde content compared to control group (Table 3).

Effects on reduced glutathion (GSH) level in mice brain tissue

The aqueous extract of *D. triflorum* 400 and 800 mg/kg dose showed significant ($p<0.01$ and $p<0.001$) increases in brain GSH level compared to control (Table 4).

Discussion

The results of the present study demonstrate that aqueous extract of *Desmodium triflorum* (L.) DC., Fabaceae, possessed anticonvulsant activity. AEDT dose of 400 and 800 mg/kg significant delayed the onset of convulsion, significantly reduced the duration of convulsion and no mortality was found of the mice against pentylenetetrazole-induced convulsion. The standard anti-epileptic drug diazepam (5 mg/kg) completely antagonized the seizures produced by pentylenetetrazole. PTZ may be exerting convulsant effect by inhibiting the activity of GABA at GABA-A receptors (Vasconcelos et al., 2007). GABA is a major inhibitory neurotransmitter in the brain, and the inhibition of its neurotransmission has been thought to be the underlying factor in epilepsy

Table 1. Effect aqueous extract of *Desmodium triflorum* on the pentylenetetrazole-induced convulsion in mice.

Experimental group	Dose mg/kg b.w.	Onset of clonic convulsion (min.)	Duration of convulsion (min)	Mortality/used (%)
Control (2% tween 80)	2.0 mL/kg (<i>p.o.</i>)	1.08±0.10	4.79±1.03	3/6 (50%)
Standard (Diazepam)	5.0 mg/kg (<i>i.p.</i>)	0.00±0.00***	0.00±0.00***	0/6 (0%)
AEDT	400 (<i>p.o.</i>)	1.88±0.48	2.64±0.67	2/6 (33.33%)
AEDT	800 (<i>p.o.</i>)	3.26±0.50*	1.99±0.54*	0/6 (0%)

All values expressed as mean±SEM; n=6 mice in each group, by one-way ANOVA followed by Dunnett's Multiple Comparison Test (compared with control group) * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

Table 2. Anticonvulsant effect aqueous extract of *Desmodium triflorum* on the MES-induced convulsion in mice.

Experimental group	Dose in mg/kg b.w.	Time in seconds of various phase of convulsion				Mortality/ used (%)
		Flexion	Extension HLTE	Stupor	Recovery	
Control (2% tween 80)	2.0 mL/kg (p.o.)	17.33±2.24	13.83±1.07	142.5±7.50	185.0±5.00	4/6 (66.66%)
Standard enytoin)	25 mg/kg (i.p.)	9.00±1.36**	-	-	11.50±1.17	0/6 (0.0%)
AEDT	400 (p.o.)	9.50±0.76**	9.50±0.76	125.5±9.32	165.0±5.40	2/6 (33.33%)
AEDT	800 (p.o.)	8.83±1.57**	9.66±0.66*	63.60±21.04	118.3±9.38*	0/6 (0.0%)

All values expressed as mean±SEM; n=6 mice in each group, by one-way ANOVA followed by Dunneet's Multiple Comparison Test (compared with control group) * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

Table 3. Effect aqueous extract of *Desmodium triflorum* on brain lipid peroxidation.

Experimental group	Dose mg/kg b.w.	Lipid peroxidation n moles of MDA/mg of protein	Decrease in MDA (%)
Control (2% tween 80) + PTZ	2.0 mL/kg (p.o.)	0.558± 0.019	0.00
AEDT+PTZ	400 (p.o.)	0.326± 0.011***	41.57
AEDT+PTZ	800 (p.o.)	0.310±0.019***	44.45

All values expressed as mean±SEM; n=6 mice in each group, by one-way ANOVA followed by Dunneet's Multiple Comparison Test (compared with control group) * $p<0.05$, ** $p<0.01$, and *** $p<0.001$.

Table 4. Effect of aqueous extract of *Desmodium triflorum* on glutathione (GSH) level in mice brain tissue.

Experimental group	Dose mg/kg b.w.	Glutathione	Increase in GSH (%)
		µmoles/mg of protein	
Control (2% tween 80) + PTZ	2.0 mL/kg (p.o.)	9.95±0.16	0.00
AEDT+PTZ	400 (p.o.)	10.82±0.17**	8.74
AEDT+PTZ	800 (p.o.)	13.07±0.18***	31.35

All values expressed as mean±SEM; n=6 mice in each group, by one-way ANOVA followed by Dunneet's Multiple Comparison Test (compared with control group) * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

(Silambujanaki et al., 2010). The enhancement of the GABAergic neurotransmission is reported to antagonize seizures, while the inhibition of the neurotransmission promotes seizures (Amabeokua et al., 2007). The protection of mice against PTZ-induced seizures by the standard anticonvulsant drugs, phenobarbitone and diazepam is expected, since various authors have shown that they exert their anticonvulsant activities by enhancing GABA-mediated inhibition (Mahomed & Ojewole, 2006).

The findings of the present study, therefore, tend to suggest that *D. triflorum* aqueous extracts have anticonvulsant activity might have inhibited and/or attenuated PTZ -induced seizures of the mice used by enhancing, or in some ways interfering with GABAergic neurotransmission.

The maximal electroshock induced convulsion in animals represents grandmal type of epilepsy. The tonic extensor phase is selectively abolished by the drugs effective in generalized tonic clonic seizure. The most outstanding action of phenytoin showed abolition of tonic extensor phase of MES seizure many drugs that increase the brain content of Gama amino butyric acid (GABA)

have exhibited anticonvulsant activity against seizures induced by MES (Manigauha et al., 2009). There are numerous molecular mechanisms through which drugs can block seizure spread and or elevate seizure threshold. Indeed, attempts to correlate the anticonvulsant profiles of antiepileptic drugs with specific mechanisms of action reveal certain notable trends. MES induced tonic extension can be blocked by drugs such as phenytoin, carbamazepine, lamotrigine, felbamate and valproate that inhibit voltage-dependent Na⁺ channels (Ambavadea et al., 2009).

The extract caused significant decrease in the duration of hind limb tonic extension (HLTE) induced by maximal electroshock. AEDT dose of 400 and 800 mg/kg significantly reduced the duration of HLTE and reduced the mortality. The standard antiepileptic drug phenytoin (25 mg/kg i.p.) significantly reduced the duration of HLTE of MES-induced convulsion and completely abolished the various phases of convulsion. Reduction in the duration of tonic hind limb extension of MES induced convulsion indicated AEDT has anticonvulsant activity. Free radicals have been suggested to be the most likely candidates responsible for producing the neuronal changes mediating

the behavioral deficits in neurodegenerative. Antioxidants are effective in rodent models of epilepsy, stroke and Alzheimer's disease (Golechha et al., 2010). Therefore the effect of AEDT on oxidative stress in PTZ induced convulsion was also evaluated. Mice pretreated with AEDT 400 and 800 mg/kg dose showed significant decrease in malondialdehyde content in mice brain compared with the control group. Glutathione is an endogenous antioxidant form within the free radicals and prevents the generation of hydroxyl radicals, the most toxic form of free radicals (Golechha et al., 2010). The decreased the level of reduced glutathione in control group mice seen in the present study indicates that there was an increased generation of free radicals and that the reduced glutathione was depleted during process of combating oxidative stress (Schulz et al., 2000). AEDT 400 and 800 mg/kg dose showed significant increases the GSH level in mice brain. The decreases in MDA and increase in the glutathione level in AEDT+PTZ mice indicates that AEDT exerted good antioxidant effect. Several studies also indicates that on medicinal plants antioxidant activities are due to the presence of polyphenols and flavonoids (Asuntha et al., 2010).

Phytochemical studies indicates the presence of phenolic compounds and flavonoids in *D. triflorum*. Phytochemical evaluation of *D. triflorum* revealed the presence of alkaloid, steroid, flavonoids, saponin, proteins and tannins in AEDT. Various phytochemicals have been reported to possess CNS activities. The anticonvulsant activity was attributed, alkaloids (Taesotikul et al., 1998), essential oils (Dallmeier & Carlini, 1981), flavonoids (Asl et al., 2007), triterpenic steroids and triterpenoidal saponins are reported to possess anticonvulsant activity in some experimental seizure models such as MES and PTZ (Chauhan et al., 1988; Kasture et al., 2002). It is also found that many flavonoids could act as benzodiazepine-like molecules in the central nervous system and modulate GABA-generated chloride currents in animal models of anxiety, sedation and convulsion (Asl et al., 2007). In the present investigation, the anticonvulsant activity can be attributed to the presence of alkaloids, steroids, flavonoids, tannins and saponin in aqueous extract of *D. triflorum*.

Conclusion

The aqueous leaf extract of *Desmodium triflorum* (L.) DC., Asteraceae, demonstrated potential anticonvulsant properties and less toxicity in the experimental animals at the doses used. However, further studies still needed to be carried on exposure of the extract to humans, and its use in folk medicine for seizure control should be accompanied by regular assessment of level of consciousness and blood pressure.

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