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"Evaluation Of Anti-Convulsant Activity Of Aqueous Extract Of Argyreia Nervosa Against Induce By Mes And Ptz Methods In Mice"

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Abstract: *The present investigation was aimed to study an anticonvulsant activity of aqueous extract of Argyreia nervosa (family: convolvulaceae) in mice. Valuated the anti convulsant activity followed by two animal models: Maximal electroshock seizures induced seizure model and Pentylenetetrazole induced seizure model. Estimation was done by measuring decrease in the duration of HLTE induced by MES, when compared with the control group. AEAN at dose of 250 & 500 mg/kg p.o. showed reduction in HLTE and no mortality was found against MES induced convulsion. Phenytoin (25 mg/kg i.p.) significantly reduced the duration of MES-induced HLTE (***p<0.001) and completely prevented the various phases of convulsion induced by MES. PTZ (60 mg/kg, i.p.) produced convulsion in all the animals used. Mice pretreated with AEDT at the dose of 250 mg/kg p.o. not showed significant delay the onset of convulsion and duration of PTZ induced seizures in mice and reduced mortality to 66.7%. Similarly a dose of 500 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 seen any mortality. Phytoconstituents like carbohydrates, flavonoids, proteins, saponins and tannins etc. AEAN at 500 mg/kg showed significant reduction of convulsions in PTZ induced petit mal epilepsy*.

Key words: *AEAN: Aquouse leave extract Argyreia nervosa, HLTE: hind limb tonic extension MES: Maximal electroshock seizures, PTZ :Pentylenetetrazole*

I. Introduction

A seizure (from the Latin sacire—to take possession of) is the clinical manifestation of an abnormal, excessive, hypersynchronous discharge of a population of cortical neurons. Epilepsy is a disorder of the central nervous system characterized by recurrent seizures unprovoked by an acute systemic or neurologic insult. Epileptogenesis is the sequence of events that turns a normal neuronal network into a hyperexcitable network [1].Recognizing the distinction between seizures and epilepsy is essential. Seizure: The clinical manifestation of an abnormal and excessive excitation and synchronization of a population of cortical neurons. Epilepsy: A tendency toward recurrent seizures unprovoked by any systemic or acute neurologic insults. Epileptogenesis: Sequence of events that converts a normal neuronal network into hyperexcitable network [1].

Seizures are brief episodes of involuntary shaking which may involve a part of the body (partial) or the entire body (generalized) and sometimes accompanied by loss of consciousness and control of bowel or bladder function. The episodes are a result of excessive electrical discharges in a group of brain cells. Different parts of the brain can be the site of such discharges. Seizures can vary from the briefest lapses of attention or muscle jerks, to severe and prolonged convulsions. Seizures can also vary in frequency, from less than one per year to several per day. One seizure does not signal epilepsy (up to 10% of people worldwide have one seizure during their lifetimes) [1].

Epilepsy is defined by two or more unprovoked seizures. Epilepsy is one of the world's oldest recognized conditions. Fear, misunderstanding, discrimination and social stigma have surrounded epilepsy for centuries. Some of the stigma continues today in many countries and can impact the quality of life for people with the disorder and their families [2].

According to WHO Epilepsy is a chronic non communicable disorder of the brain that affects people of all ages. Around 50 million people worldwide have epilepsy. Nearly 80% of the people with epilepsy are found in developing regions. Epilepsy responds to treatment about 70% of the time, yet about three fourths of affected people in developing countries do not get the treatment they need. People with epilepsy and their families can suffer from stigma and discrimination in many parts of the world [2].

II. Materials And Methods:

Animals:

Albino mice of either sex of average weight 25-30gm aged 3-4 months were housed in clean polypropylene cages with 12 h light/dark cycle at 25±2ºC and 65±5% humidity. They had access to food

(standard pellet diet, Hindustan Lever Ltd) and water. All experiments were carried out between 11 AM and 3 PM. The ethical clearance was obtained from the Institutional Animals Ethical Committee and all the experiments have been carried according to CPCSEA guidelines.

Chemicals and Drugs:
All chemicals and drugs used were of commercial grade. These included PTZ, diazepam, and phenytoin.

Collection and Identification:
The plant leaves of Argyreia nervosa were collected locally from Acharya nagrjuna University campus, Guntur district of Andhra Pradesh. The plant was identified, confirmed and authenticated by comparing with voucher specimen available at survey of Medicinal plants and collection unit, Department of Botany Acharya Nagarjuna University.

Preparation of Aqueous Plant Extract:
The leaves were cut into small pieces, powdered and the crude drug macerated with distilled water for 24 hours. Then it is filtered and the solvent was removed by freeze dryer and stored in vacuum desiccators, which was used for in-vivo anti-convulsant investigations.

Phytochemical Screening:
Preliminary qualitative phytochemical screening was done for flavonoids [Shinoda test], saponins [froth formation test] and Tannins [color reaction with ferrous chloride] as per standard methods.

Acute Toxicity Studies :
The acute toxicity study was conducted as per the OECD guidelines 423 (OECD, 2001) by Kamal et al., 2012, where the limit test dose of 2000 mg/kg was used and reported that extract was safe upto 200 mg/kg.

Anticonvulsant Activity

1. Maximum electro shock (MES) induced convulsions:
The mice were divided into 4 groups consisting of 6 animals each group.
- GROUP I: Treated with 1% acacia (p.o.)
- GROUP II: Treated with phenytoin (25mg/kg, i.p.)
- GROUP III: Treated with extract (250 mg/kg, p.o.)
- GROUP IV: Treated with extract (500 mg/kg, p.o.)

The electroshock was applied via ear-clip electrodes separately to each mouse. The stimulus duration was 0.2 s and the current frequency 30 mA. Six mice of either sex in one group with a weight of 25±5 g. Test was started 30 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 [3].

2. Pentylenetetrazole (PTZ) induced convulsion:
The animals were divided into 4 groups each contain 6 mice.
GROUP I: Treated with1% Acacia (p.o.)
GROUP II: Treated with Diazepam (5 mg/kg, i.p.)
GROPU III: Treated with extract (250 mg/kg, p.o..)
GROUP IV: Treated with extract (500 mg/kg, p.o.)

Animals were divided into IV groups, (n=6 mice of either sex in one group). Mice were administered with PTZ (60 mg/kg i.p), 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 [3].

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

III. Results

Preliminary phytochemical screening

The percentage yield of the aqueous extract of A. nervosa leaves was found to be 4% w/w. Qualitative test showed the presence of carbohydrates, flavonoids, proteins, saponins and tannins etc., but not found alkaloids.

Table 1: Phytochemical screening of extract

S.No.	PHYTOCHEMICALS	TESTS	AEAN
01	ALKALOIDS	Dragendroff's test	-
		Mayer's test	-
		Hager's test	-
		Wagner's test	-
		Picric acid test	-
02	CARBOHYDRATES	Molisch's test	+
		Fehling's test	-
		Benedict's test	-
03	SAPONINS	Foam Test	+
04	FLAVNOIDS	Alkaline Reagent test	+
		Lead acetate test	+
		Shinoda test	+
06	TANNINS	Acetic acid test	+
07	PHENOLICS	Ferric Chloride test	+
08	AMINO ACID/PROTEINS	Biuret test	+
		Ninhydrin test	+

Present=+, Absent=-

Acute oral toxicity

Previous reports on acute toxicity study showed no mortality and unusual effects at a dose of 2000 mg/kg.

Anti-Convulsant Activity

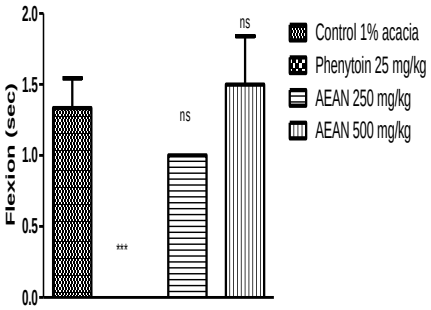
Effect of extract on MES-induced Seizures

The aqueous extract of AEAN caused significant decrease in the duration of hind limb tonic extension (HLTE) induced by maximal electroshock, when compared with the control group. AEAN at dose of 250 & 500 mg/kg p.o. showed reduction in HLTE and no mortality was found against MES induced convulsion. Phenytoin (25 mg/kg i.p.) significantly reduced the duration of MES-induced HLTE (***p<0.001) and completely prevented the various phases of convulsion induced by MES (Table 3, fig. 2). AEAN showed minimal inhibition of MES induced seizures, when compared to phenytoin.

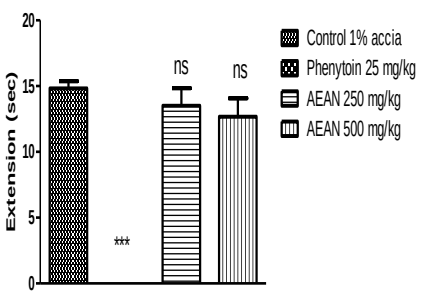
Table 2: Anticonvulsant effect aqueous extract of Argyreia nervosa on the MES-induced convulsion in mice

Experimental groups	Dose in mg/kg b.w.	Time in seconds of various phase of convulsion				Survivors / used
		Flexion	Extension	Clonic	Stupor	
Control(1% Acacia)	1 ml/kg (*p.o.*)	1.33±0.17	14.83±0.54	10.83±2.81	27.5±003	6/6
Phenytoin	25 mg/kg (*i.p.*)	0±0.0	0±0.0	15.5±2.23	0±0.0	6/6
AEAN	250 mg/kg (*p.o.*)	1±0.0	13.5±1.33	8.83±0.60	17.33±2.17	6/6
AEAN	500 mg/kg (*p.o.*)	1.5±0.28	12.66±1.40	6±1.39	3.66±1.03	6/6

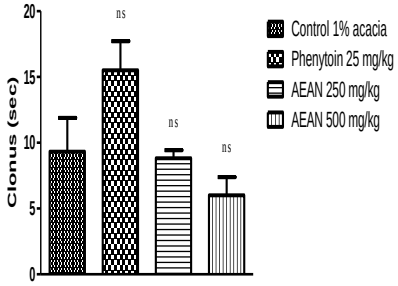
All values expressed as mean±SEM; n=6 mice in each group, by one-way ANOVA followed by Dunneet's Multiple Comparison Test (compared with control group) *p<0.05, **p<0.01, ***p<0.001 and ns-no significance.

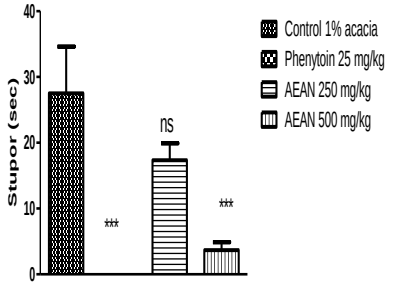


A. Flexion



B. Extension





C. Clonus **D. Stupor**

Fig 1: Consolidated graphs representing the duration of four stages of convulsions in MES (sec) A. Flexion, B. Extension, C. Clonic & D. Stupor. 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, ***p<0.001 and ns- no significance.

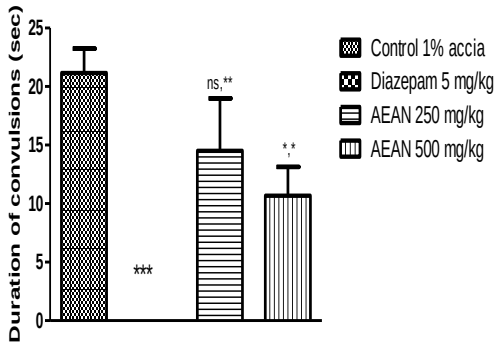
Effect of extract on pentylenetetrazole induced Seizures

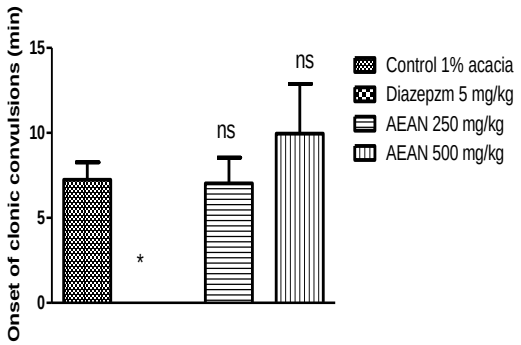
Pentylenetetrazole (60 mg/kg, i.p.) produced convulsion in all the animals used. All extracts groups compared with the control group. Mice pretreated with AEDT at the dose of 250 mg/kg p.o. not showed significant delay the onset of convulsion and duration of pentylenetetrazole induced seizures in mice and reduced mortality to 66.7%. Similarly a dose of 500 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 3, fig. 3).

Table 3. Effect of aqueous extract of Argyreia nervosa on PTZ-induced seizure in mice

Experimental groups	Dose in mg/kg b.w.	Onset of clonic (min)	Duration (sec)	Survivors/ used	% protection against mortality after 30 min
Control (1% acacia)	1 ml/kg (*p.o.*)	7.23 ± 1.03	21.17± 2.07	4/6	66.7
Diazepam	5 mg/kg (*i.p.*)	0.0±0.0***	0.0±0.0***	6/6	100
AEAN	250 mg/kg (*p.o.*)	7.03±1.51	14.50±4.45	4/6	66.7
AEAN	500 mg/kg (*p.o.*)	9.96 ± 2.915	10.67±2.45	6/6	100

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, ***p<0.001 and ns-no significance.





A. Duration of convulsions **B. Onset of clonic convulsions**

Fig 2. Consolidated graphs representing the A. duration of convulsions & B. Onset of clonic convulsions in PTZ induced model. 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, ***p<0.001 and ns-no significance.

IV. Discussion

Epilepsy is a common health problem throughout the world. Patients compliance with Antiepileptic drugs is a major problem because of the need for long-term therapy together with adverse effects of many drugs. The search for new therapies with better efficacy and tolerability remains to be an important area of research. The discovery and development of a new AED's depends heavily on the preclinical use of animal models to establish efficacy and safety prior to clinical trials. Among the animal model tests used for evaluation of anticonvulsant activity, the MES and PTZ tests are of predictive relevance regarding the clinical spectrum of activity of experimental compounds, since the MES and PTZ tests are assumed to identify anticonvulsant drugs effective against human generalized tonic-clonic and absence seizures, respectively [7, 8].

In MES model, substantia nigra is the only definite structure found to be involved in the seizures. MES produces generalized tonic clonic seizures, which can be prevented either by drugs that inhibit voltage-dependent Na+ channels such as phenytoin, Na+valproate, felbamate and lamotrigine; or by drugs that block glutamatergic receptor such as felbamate[9].

PTZ-induced seizures appear to be mediated by an extensive neural system involving the reticulate formation, diencephalic region and caudal hypothalamus. PTZ produces absence seizures (generalized clonic seizures) can be treated by drugs that reduce T-type Ca++ channels, such as ethosuximide can prevent seizures induced by PTZ. Drugs like benzodiazepines and phenobarbitone prevent generalized clonic seizures by enhancing gamma amino butyric acid type A (GABAA) receptor mediated inhibitory neurotransmission, and perhaps valproate and felbamate can treat this type of seizure[9].

Current available anticonvulsant drugs are able to efficiently control epileptic seizures in about 75% of the patients. Furthermore, adverse effects from the drugs used clinically often make treatment difficulty; so there are demands for new types of anticonvulsants drugs. One of the approaches to search for new antiepileptic drugs is the naturally occurring compounds, which may belong to new structural classes [10].

The results of this study demonstrated that the aqueous extract A. nervosa have anticonvulsant activity against PTZ induced seizures. Data showed that the AEAN at 250 & 500 mg/kg delayed onset of first convulsion and reduced duration of clonic convulsions, compared to control group. AEAN at 250 mg/kg and 500 mg/kg showed 66.7% & 100% protection against mortality induced by PTZ, respectively. Therefore, AEAN showed protective effect against petit mal epilepsy.

In MES model extract showed delayed extension, clonus, stupor and showed 100% protection against 30 mA for 0.2 sec. AEAN at the dose of 250 and 500 mg/kg showed minimal reduction in the HLTE duration compared to control group. 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. AEAN showed significant reduction in duration of clonic convulsions in MES model, when compared to control and standard phenytoin. AEAN did not show anticonvulsant activity in MES induced grand mal epilepsy. Thus, A. nervosa has no protective effect against the grand mal epilepsy. As this plant exhibited more antiepileptic activity in the PTZ induced seizure test than in MES test, it could be more useful against petit mal epilepsy.

The anticonvulsant activity of A. nervosa may be attributed to the presence of flavoniods and tannins, which have been found in its aqueous extract by phytochemical investigation. The aqueous extract Phytochemical studies indicate the presence of phenolic compounds, flavonoids and saponins in A. nervosa. The anticonvulsant activity of triterpens [11], flavonoids [12], saponins [13-14] and alkaloids [15] has been demonstrated previously. 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 [16]. Flavonoids are a class of natural constituents that are widely distributed in plants with many pharmacologic properties including antiseizure properties. Literature reports on flavonoids revealed that, they were able to prevent the expression of tonic–clonic seizures induced by PTZ through central benzodiazepine (BZD) receptors. In the present investigation, the anticonvulsant activity can be attributed to the presence of flavonoids, tannins and saponin in aqueous extract of A. nervosa.

V. Conclusion

The anticonvulsant effect of AEAN was confirmed by following measures. The AEAN demonstrated anticonvulsant activity by dose dependent manner. The acute toxicity study indicated that the AEAN is devoid of major toxic effects. PTZ and MES models were used for induction of convulsions in mice. The AEAN at 500 mg/kg showed significant reduction of convulsions in PTZ induced petit mal epilepsy. Further studies will be necessary to establish the probable mechanism of action of plant extracts of AEAN. The present investigation has also opened avenues for further research especially with reference to the isolation and development of potent phytomedicine for treatment of convulsions.

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