EVALUATION OF ANTICONVULSANT AND ANXIOLYTIC ACTIVITY OF ETHANOLIC EXTRACT OF ARGYREA NERVOSA

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ABSTRACT

Argyreia speciosa commonly known as *Vridha daraka* in Sanskrit is one of the important plants used in indigenous system of medicine. The root is regarded as an alternative tonic and useful in the diseases of nervous system. To confirm the veracity of aforementioned claim, we have evaluated the anticonvulsant effect of the extract. In this investigation, the mice were pre-treated with different doses of *Argyreia speciosa* extract (100, 200, 400 mg/kg) for 10 days and then, they were subjected to either pentylenetetrazole (80 mg/kg) or maximal electroshock seizures (50 mA, 0.2 s) treatment. The hydro alcoholic extract of *Argyreia speciosa* at the dose of 200 and 400 mg/kg significantly delayed the latency to the onset of first clonus as well as onset of death in unprotected mice and exhibited protection in 16.66% and 33.33% of pentylenetetrazole treated mice respectively. Whereas in case of maximal electroshock-seizures, the dose of 200 and 400 mg/kg significantly reduced the duration of hind limb extension and both the doses were statistically found to be equipotent. The reference standards, clonazepam (0.1 mg/kg) and phenytoin (20 mg/kg) provided complete protection. Thus, present study revealed anticonvulsant effect of *Argyreia speciosa* against pentylenetetrazole- and maximal electroshock- induced convulsions in mice.

Materials and Methods

Collection of Plant Materials

The whole plant of *Argyrea nervosa* was collected from Tirumala hills, Tirupathi, Andhra Pradesh, India. The plant was identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh, India, and voucher specimen has been deposited in the departmental herbaria.

Preparation of extract

The whole plant species was collected and then dried under shade for a period of four weeks. The dried plant material (500g) was milled to a fine powder using commercial laboratory blender. The dried powder (300g) was extracted in a Soxhlet extractor with Ethanol. The extraction was continued until the solvent in the thimble became clear. After complete extraction, the extract was filtered and the solvent was distilled off. Then it was concentrated at 40^0 C under reduced pressure using Buchi R-153 Rotavapour to obtain the dry residue. The yield of the crude Ethanol extract was 30g. The extract was the stored in desiccators until use.

Drugs and Chemicals

Drugs and chemicals used in the study were obtained commercially and were of analytical grade. Phenytoin and Pentylenetetrazole (Sigma, USA), Isoniazid (Novartis India ltd., Hyderabad, India), DMSO and Ethanol (Hi-pure fine Chem Industries, Hyderabad, India).

Animals

For the screening of antiepileptic activity, studies were carried out using Swiss albino mice (18-22g) and Wistar albino rats (150-180 g) of either sex. All the animals were procured from Sainath Agencies, Hyderabad, India for experimental purpose. After procuring, all the animals were acclimatized for 7 days and housed in groups of six under standard laboratory conditions, like room temperature 26 ± 2^0C, relative humidity 45-55% and light/dark cycle of 12h. All the animals were provided with synthetic standard diet and water was provided *ad libitum* under strict hygienic conditions. Animal experimentation protocols are approved by Institutional Animal Ethical Committee (IAEC) of GSN Pharmaceuticals Pvt. Ltd., Kukatpally, Hyderabad, India.

Preliminary Phytochemical Screening

The preliminary phytochemical investigations were carried out with the ethanolic extract of Argyrea nervosa whole plant for qualitative identification of phytochemical constituents using standard conventional protocol. All the chemicals and reagents used were of analytical grade [1-3].

Acute Toxicity Study

The acute toxicity of the Ethanolic extract of Argyrea nervosa whole plant was determined as per the OECD guideline no. 423 (Organization for Economic Co-operation and Development). It was observed that the test extract was not mortal even at a dose of 2000 mg/kg body weight. Hence, 200 mg/kg, 400 mg/kg and 600 mg/kg doses were selected for further study.

Antiepileptic activity

Isoniazid-induced epileptic seizure model:

Swiss albino mice (18-22 g) of either sex were divided into V groups of six animals each. Group I served as control and was administered 10% (w/v) DMSO (5 ml/kg, p.o.). Group II was administered phenytoin (5 mg/kg, i.p) on the first day alone and served as standard group. Groups III, IV and V were treated with different doses of ethanolic extract of *Argyea nervosa* whole plant (200 mg/kg, 400 mg/kg and 600 mg/kg, p.o.) respectively once daily for seven days. On the seventh day 60 min after control, standard and extract administration into respective groups, Isoniazid (300 mg/kg s.c) was administered. The following parameters were recorded during test session of initial, 30 min and upto 24 h. The animals were observed for latency (onset of epileptic seizure), status of animal after 30 min, status of animal after 24 h period and the percentage [4].

Pentylenetetrazole-induced epileptic seizure model:

The seizure was induced by administration of pentylenetetrazole (80 mg/kg i.p) to Wistar **albino rats** of either sex. The rats showing response were divided into IV groups of six animals each. Group I was administered 10%(w/v) DMSO (5 ml/kg, p.o.) which served as control. Group II was allotted for standard drug where animals were treated with Phenytoin (25 ml/kg, i.p) [25]. Group III and IV were treated with different doses of ethanolic extract of

Argyrea nervosa whole plant (200mg/kg and 400 mg/kg, i.p) respectively. The doses were given for seven days as multiple dose studies. Animals were fasted overnight prior to the test but water was supplied *ad libitum*. Drug pretreatment was given 1 hr prior to the administration of pentylenetetrazole, then animals were placed in plastic cages individually and were observed for the duration of tonic flexion, tonic extension, clonus, death or recovery and percentage protection of the animal, initially for 30 min and later upto 24 hr period.

2.4 Assessment of anxiolytic activity

2.4.1 Elevated plus maze

The **anxiolytic activity** was assessed using elevated plus maze (EPM) as described by Pellow [5]. In brief, the EPM consisted of two open arms (25x5cm) crossed with two enclosed arms (25x5x20cm) The arms were connected with a central square of 5x5cm. The apparatus was elevated to the height of 25cm in a dimly illuminated room. The mice treated with the extract (10-100mg/kg) or vehicle were placed individually in the center of the EPM facing an enclosed arm and the time spent in open and closed arm was recorded for 5min.The number of entry in the enclosed arm was also noted during this time. An entry was defined as all four paws in the arm. The ratio of time spent in open versus closed arm was calculated. The EPM was cleaned with hydrogen peroxide after each trial.

Statistical Analysis

The results were expressed as mean ± SEM and statistically analyzed by one-way ANOVA followed by Tukey- krama test. The results obtained were compared with the control group. *p* values < 0.05 were considered to be statistically significant (*p* denotes probability)[6].

RESULTS

Phytochemical screening

The qualitative analysis of EEAN showed the presence of various phytoconstituents such as alkaloids, steroids, tannins, flavonoids, glycosides, sesquiterpenes, proteins and amino acids (Table 1).

Table-1: Results of Phytochemical Screening of the ethanolic extract of *Argyrea nervosa* whole plant (EEAN)

Phytoconstituents	EEAN
Reducing sugars	-
Alkaloids	+
Tannins	+
Flavonoids	+
Glycosides	+
Phytosterols	+
Triterpenoids	+
Proteins and Amino acids	+

+: Positive result ; - : Negative result

Antiepileptic activity

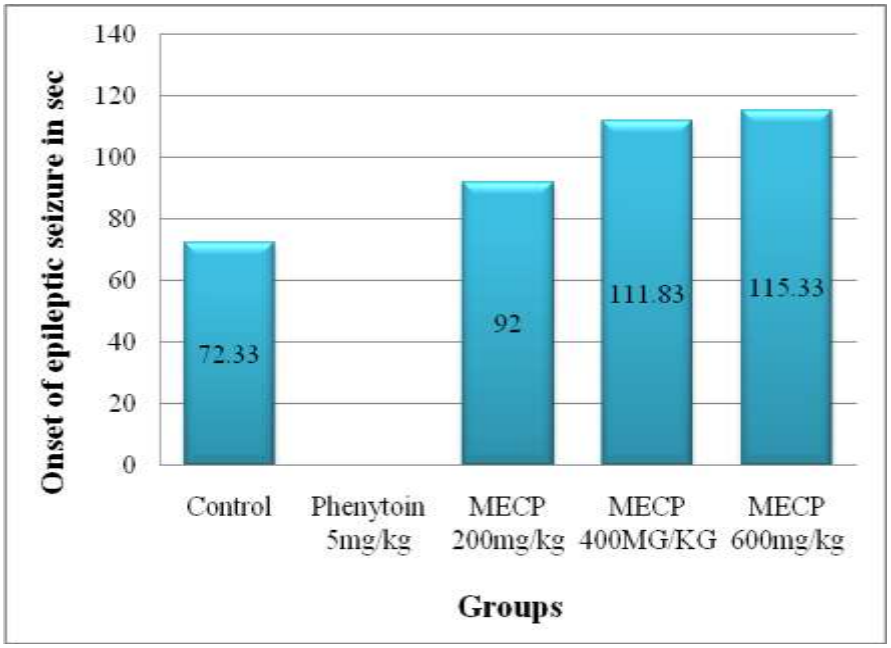
INH- induced epileptic seizure in mice:

The antiepileptic activity of the ethanolic extract of *Argyrea nervosa* whole plant using INH-induced epileptic seizure in mice is expressed in (Table 2). In this test the onset of epileptic seizure in the control group occurred at 72.33±0.75 sec and the extract treated groups at doses 200 mg/kg, 400 mg/kg and 600 mg/kg significantly ($p< 0.00$) delayed the onset of epileptic seizure time to 92.0±0.57 sec, 111.83±0.79 sec and 115.33±0.49 sec respectively (Graph 1). The standard antiepileptic drug, phenytoin 5mg/kg i.p totally abolished the effects of INH-induced epileptic seizures in mice with 100% protection, whereas the various doses of EEAN at 200mg/kg, 400mg/kg and 600 mg/kg showed the percentage protection of 50%, 33% and 50% respectively.

Table-2: Effect of ethanolic extract of *Argyrea nervosa* whole plant on INH-induced epileptic seizure in Mice

Groups	**Treatment**	**Dose (kg^{-1})**	**Latency(onset of epileptic seizure in sec)**	**Status of animal after 30 min(no.of animals alive)**	**Status of animal after 24 hr (no.of animals alive)**	**Percentage protection**
I	Control (10% w/v DMSO) *p.o*+INH *s.c*	5ml+300mg	72.33±0.75	4	1	16
II	Phenytoin *i.p* + INH *s.c*	5mg+300 mg	NIL**	6	6	100
III	EEAN *p.o*+INH *s.c*	200mg+300 mg	92.0±0.57	4	3	50
IV	EEAN *p.o*+INH *s.c*	400mg+300 mg	111.83±0.79 *	3	2	33
V	EEAN *p.o*+INH *s.c*	600kg+300 mg	115.33±0.49 *	3	3	50

*Values are expressed as Mean±SEM (Standard Error Mean); Values are calculated as compared to control using one way-ANOVA followed by Tukey-kramer test, *indicates $p<0.05$, **indicates $p<0.01$vs. control; n=6; p.o.: per oral; s.c: subcutaneous; i.p.: intraperitoneal route of administration.*



Graph-1: Effect of EEAN on Onset of epileptic seizure in mice

PTZ-induced epileptic seizure in rats:

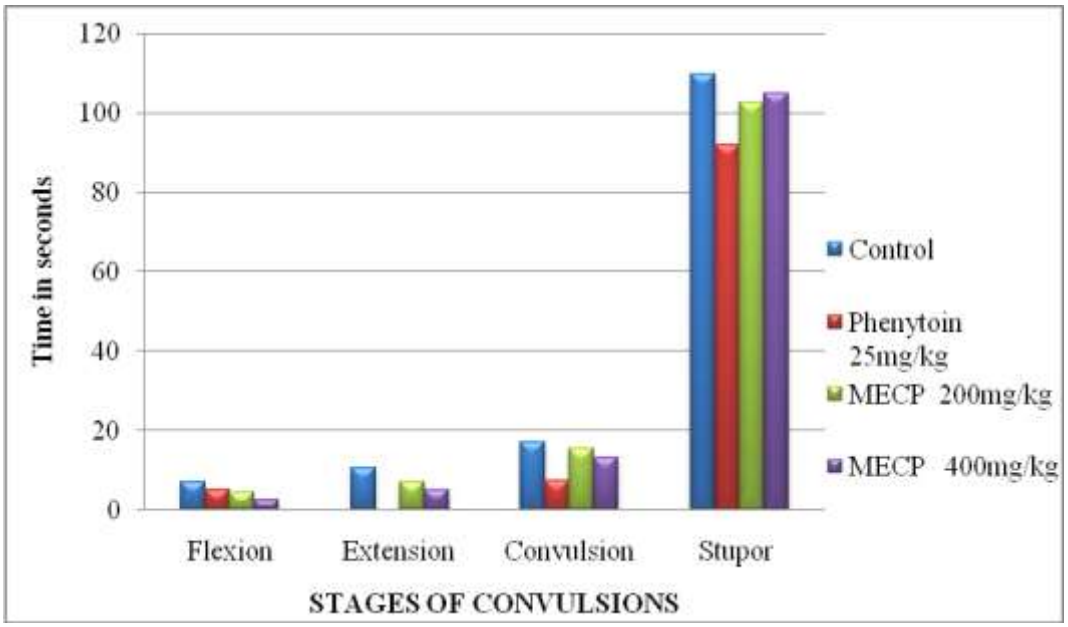
Table 3 showed the antiepileptic activity of the ethanolic extract of Argyrea nervosa whole plant using PTZ-induced epileptic seizure in rats. EEAN exhibited significant antiepileptic activity by lowering the duration of extension phase when compared with the control group. The duration of tonic and hind limb extension in rats with the extract treated groups at doses 200 mg/kg and 400 mg/kg was 7.16 ± 0.67 and 5.0 ± 0.99 respectively. The ethanolic extract of Argyrea nervosa whole plant at different doses was comparable ($p<0.00$) with that produced by standard drug Phenytoin 25 mg/kg. The different stages of epileptic seizure *vs* Time in seconds is shown in Graph 2

Table-3: Effect of ethanolic extract of Argyrea nervosa whole plant on PTZ- induced epileptic seizure in Rats

Groups	**Treatment**	**Dose (kg^{-1})**	**Time in seconds of various phases of convulsions**				**Recovery**	**% protection**
			Flexion	**Extension**	**Convulsion**	**Stupor**		
I	Control(10% DMSO) *p.o*+ PTZ *i.p*	5ml+80 mg	7.16±0.47	10.6±0.34	17.1±0.30	109.6±0.494	192.24	0

II	Phenytoin *i.p*+ PTZ *i.p*	25mg+80mg	5.16±0.36	0**	7.5±0.76**	91.8±0.401**	93.5	100
III	EEAN *i.p*+PTZ *i.p*	200mg+80 mg	4.33±0.66*	7.16±0.67*	15.33±0.42	102.5±0.428*	156.79	70.93
IV	EEAN *i.p* +PTZ *i.p*	400mg+80 mg	2.66±0.49**	5.0±0.99**	13.16±0.47*	104.8±0.421	134.51	83.04

*Values are expressed as Mean±SEM (Standard Error Mean); Values are calculated as compared to control using one way-ANOVA followed by Tukey-kramer test, *indicates p<0.05, **indicates p<0.01 vs. control; n=6; p.o.: per oral ; i.p.: intraperitoneal route of administration.*



Graph-2: Effect of EEAN on stages of convulsions in PTZ-induced epileptic seizure in Rat.

Table 04: Anti-anxiety activity of various extracts of Argyrea nervosa in elevated plus maze model.

S.No	treatment	Dose (mg/kg)	Mean number of entries in open arms	Mean time(sec) spent in open arms S.E.M

			S.E.M	
1.	Control	Vehicle	2.4 ± 0.45	2.91 ± 0.28
2	Diazepam	2.0	7.2 ± 0.42	14.53 ± 0.29
3	EEAN	100	3.4 ± 0.38	3.73 ± 0.47
4	EEAN	200	6.8 ± 0.83	13.46 ± 1.04
5	EEAN	400	3.3 ± 0.11	3.98 ± 0.27

*Values are expressed as Mean±SEM (Standard Error Mean); Values are calculated as compared to control using one way-ANOVA followed by Tukey-kramer test, *indicates p<0.05, **indicates p<0.01 vs. control ;n=6; p.o.:per oral ; i.p.: intraperitoneal route of administration.*

DISCUSSION

In the present study the antiepileptic action of the ethanolic extract of *Argyrea nervosa* whole plant was evaluated in rodents against INH-induced epileptic seizure and PTZ-induced epileptic seizure.

Following fractionation, all of the major compounds from the whole extract were recovered in appropriate fractions, although it is not clear if this was achieved quantitatively (recovery rates were 94% for fractionation and 98% for sub fractionation). One possible explanation for the loss of anxiolytic activity of the reconstituted fractions might be a change in interaction between certain compounds from the extract due to the fractionation. The structure of kaempferol was confirmed by ^{1}H-NMR analysis. The antioxidant properties of the extract and some compounds in comparison to structurally related substances showed a high antioxidant effect of the whole extract as well as a tannin-rich extract evaluated by the oxygen radical absorbance capacity (ORAC) assay. This is in well agreement with literature sources that evaluated a variety of radical-scavenging activities of AV [7-12]. Caffeic acid derivatives, quercetin, and the glycosylated flavonoids presented with a high antioxidant activity based on structure-activity relationships as has been reported before.

Pharmacological evaluation in 3 different animal models of anxiety of the whole extract revealed a significant anxiolytic activity in two distinct dose ranges of 22.5-30 and 100-125 mg/kg comparable to the known anxiolytics diazepam and buspirone. This unique double U-shaped activity is an extension to the occurrence of U-shaped activities, which are not yet fully understood, but may be due to hormesis. Hormesis is a concept first described in toxicology where a low-dose stimulation occurred followed by a high-dose inhibition [13-14]. This phenomenon has been observed in a variety of receptor systems and hormonal responses including the dopamine and serotonin receptors as well as

corticosterone and estrogen, which all play a role in stress and anxiety disorders. Another explanation of the specific occurrence of a double U-shaped anxiolytic effect observed in AV might be the involvement of two receptor systems and a receptor selective dose-response profile. This is supported by fractionation and antagonism studies on the GABA-ergic and serotonergic receptors, where the extract and fractions were antagonized by flumazenil (GABAA antagonist) in a dose equivalent to 125 mg/kg and the fractions equivalent to 30 mg/kg of whole extract were significantly antagonized by WAY-100635 (5-HT1A antagonist).

The involvement and interconnection of GABA and serotonin as neurotransmitters for anxiety responses in neuronal circuits has been shown both *in vitro* [15] and *in vivo* [16.] Another possibility for the distinct activity of the two doses might be related to solubility limitations of active compounds, biotransformation to active or inactive metabolites, or enzyme saturation. At this point, it is not clear which of these mechanisms is responsible for the observed anxiolytic dose-response profile keeping in mind that it might even be the contribution of more than one discussed or yet not known mechanism.

The activity of AV in a dose of 30 mg/kg was observed in all three animal models, namely the Elevated Plus Maze (EPM), the Light-Dark Transition (LDT) test, and the Stress-Induced Hyperthermia (SIH) while the higher concentration of 125 mg/kg only exerted anxiolytic-like effects in the EPM. All three animal models use distinct paradigms for the induction of anxiety and evaluate different parameters related to the respective condition. In fact, the differential response of the two AV doses supports the theory of different receptor systems being targeted at the two doses. It has been suggested in the literature that the EPM and the LDT can distinguish between specific anxiety disorders [17-20]. In addition, the SIH uses a stress-related paradigm for the induction of an anxiety behavior where the animal is not able to avoid exposure to the stressful situation, which is not the case for both the EPM and the LDT [21-26].

Chronic treatment over 16 or 22 days with AV did mostly not present with a sustained significant anxiolytic effect and neither did the known anxiolytic diazepam, which was also administered. This lack of anxiolytic effect after chronic administration has been observed before and attributed to adaptive mechanisms of receptor systems, neuronal circuits, or hormonal feedback mechanisms [27-31].

CONCLUSION

In the present study, EEAN was evaluated by using various experimentally induced seizure models. EEAN at doses of 200mg/kg, p.o and 400mg/kg, p.o showed significant delay in onset of tonic convulsions and decreased the duration of seizures in PTZ, and Isoniazid induced seizures. In MES model, EEAN had significant effect in abolishing tonic hind limb extensions. From all the above findings, the present investigation suggests that the ethanolic extract of *A. nervosa* seeds may possess antiepileptic activity against PTZ, PIC, INH induced seizures by enhancing GABA inhibitory neurotransmission and MES, it blocks seizures spread and tonic extension either by inhibit voltage dependent Na+ channels or by blocking glutamatergic excitation mediated by the N-methyl- D-aspartate (NMDA) receptor.

Therefore, lend pharmacological credence to the traditional use of this plant in the treatment of epilepsy.

However, an extensive Pharmacological study of this plant is required for complete understanding of the antiepileptic activity of ethanolic extract of *A. nervosa* seeds. The confirmation of phytochemical screening gave positive results for alkaloids and flavonoids which may be the active constituent responsible for the antiepileptic activity of *A. nervosa.* Further investigation should be carried out to isolate and identify the chemical constituent which is responsible for its antiepileptic activity. In conclusion the results of the present study revealed significant antiepileptic potential of the ethanolic extract of *A.nervosa* whole plant. It is therefore possible that the antiepileptic activity of the plant may be exerted by the various phytoconstituents present in the plant *viz*., alkaloids, flavonoids, steroids, tannins, glycosides, sesquiterpenes, proteins and amino acids and justify its use as a traditional folk remedy for central nervous system

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