

EVALUATION OF POSSIBLE ANTICONVULSANT EFFECT OF *ALSTONIA SCHOLARIS* (LINN.) R. BR. EXTRACT ON EPILEPSY MODELS

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ABSTRACT**Objective:** In this study, the effects of ethanolic extract of *Alstonia scholaris* (EAS) were evaluated on epilepsy models.**Methods:** Pentylentetrazole (PTZ), pilocarpine, and maximal electroshock (MES)-induced convulsions in mice were recorded. EAS (100, 200, and 300 mg/kg, p.o.) protected of mice against PTZ, pilocarpine, and MES-induced seizures, respectively.**Results:** In MES-induced seizures, the results revealed that EAS decreased the duration of the tonic hindlimb extension and percent mortality. In addition, these doses of EAS increased the latency for the development of convulsions and reduced mortality in mice against PTZ and pilocarpine-induced convulsions.**Conclusion:** The ethanolic extract of bark *A. scholaris* exhibited anticonvulsant potential in various animal models.**Keywords:** *Alstonia scholaris*, Pentylentetrazole, Pilocarpine, Maximal electroshock, Anticonvulsant effect.**INTRODUCTION**

Epilepsy is one of the most common and extensive neurological disorders in the human population [1]. Epilepsy is characterized by the occurrence of spontaneous seizures induced by a complex of neurotransmitter systems [2]. Most epileptic syndromes contain particular neurophysiological and clinical characteristics, the seizures being the result of abnormal, hyperactive, or hypersynchronous neuronal discharges [3]. Conventional treatment of epilepsy mainly based on anticonvulsant medication. Interestingly, in the last years, a growing body of evidences has demonstrated that natural products from folk remedies contributed significantly in the discovery of modern drugs worldwide. Moreover, numerous herbal medicines are active on the central nervous system (CNS), and they have at least a hypothetical potential to affect chronic conditions such as anxiety, depression, headaches, or epilepsy that do not respond well to conventional treatments [4,5].

Plant extract can be an important source for the development of alternative and complementary treatment of epilepsy. Some plants reputed to possess antiepileptic properties in different cultures have been found to exhibit anticonvulsant activity in different animal models [6]. Plant *Alstonia scholaris* (Linn.) belonging to family apocynaceae is commonly known as Saptaparna. The plant is native to India and grows in deciduous and evergreen forests and also in the plains. The plant is known to contain alkaloids (ditamine and echitamine), flavonoids, and phenolic acids [7]. In the literature, this plant is reported as a bitter, astringent, digestive, laxative, anthelmintic, antipyretic, stomachic, cardiotoxic and tonic [8]. The bark extract has been reported to possess antiplasmodial, immunostimulant, anticancer effect and antistress activity [9]. However, there is no evidence indicated the influence of ethanolic extract of *A. scholaris* (EAS) on epilepsy models. Therefore, an effort has been made to evaluate the anticonvulsant effect of *A. scholaris* on epilepsy models.

METHODS**Animals**

Male Swiss mice (20-30 g) were purchased from the National institute of nutrition of India (Hyderabad). Animals were kept in plastic cages

in rooms with a controlled 12/12 hrs light/dark cycle, a temperature of 25°C, and food and water *ad libitum*. The animal studies were approved by the Institutional Animal Ethics Committee, constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India. The animals were removed from the vivarium to the testing area in their home cages and allowed to adapt to the new environment for at least 1-hr before testing. Testing was carried out in a counterbalanced order with respect to the treatment conditions in the noise free room.

Plant materials and preparation of the extract

The barks of *A. scholaris* were purchased in bulk from the local market and identity of the plant and analysis from Unijules life science Ltd., India. Voucher specimen (500B201) was deposited in our research laboratory for the future reference. Dried powdered barks were extracted with ethanol to obtain an ethanolic extract. The extract obtained was dried at 40°C using a vacuum evaporator.

Acute toxicity

Toxicity studies were carried out in accordance with OECD guidelines, acute oral toxicity study of EAS. The EAS (50, 100, 150, 200, 300 mg/kg/day) was administered orally for 4 days of six groups of mice (n=6) and the animals were kept under examination for mortality as well as any behavioral changes.

Chemicals

Pentylentetrazole (PTZ), pilocarpine (Hi-media Laboratories Pvt. Ltd., India), phenytoin (Pfizer, India), and diazepam (Ranbaxy, India) were used in the present study. All the chemicals were dissolved in saline.

Anticonvulsant activity**PTZ and pilocarpine-induced seizure models**

Animals were treated with EAS (100, 200 and 300 mg/kg, p.o.) or saline (control) 30 minutes before administration of PTZ (85 mg/kg, i.p.) or pilocarpine (400 mg/kg, i.p.) placed in individual cages, and observed for 60 minutes. The parameters measured were: latency to the first convulsion or death, the percentage of animals convulsing, and percentage of animals surviving [10].

Maximal electroshock (MES) - induced seizures test

Mice were divided into four groups each containing six animals and treated with either saline, EAS (100, 200, and 300 mg/kg, p.o.). 30 minutes later seizures were induced by a current stimulus (18 mA, 50 Hz for 0.2 seconds) delivered by using corneal electrodes by a shock generator. The percent protection and duration of the tonic hindlimb extension (i.e., the hind limbs of animals outstretched at 180° to the plane of the body axis) was observed. Protection was defined as the complete absence of tonic hindlimb extension [11].

Phytochemical screening

EAS was subjected to phytochemical screening [12-14] for the detection of various phytoconstituents.

Statistical analysis

All results are presented as means \pm standard error mean. ANOVA followed by Tukey's *post-hoc* test. The results were considered significant at $p < 0.05$.

RESULTS**Acute toxicity**

EAS was found to be safe at a dose of 2000 mg/kg, p.o; hence, a dose of 100, 200 and 300 mg/kg, p.o. were selected for *in-vivo* pharmacological studies.

Anticonvulsant activity**PTZ and pilocarpine-induced seizure models**

As shown in Tables 1 and 2, the seizure latency was prolonged by all the test groups as compared to vehicle treated mice. The percentage protection afforded increased at all dose levels of EAS (100, 200 and 300 mg/kg, p.o.), whereas the duration of tonic flexion and clonus of the test groups were significantly ($p < 0.01$) lowered at all dose levels. Maximum protection was achieved with diazepam (3 mg/kg, i.p.).

Table 1: Effects of *A. scholaris* extract on PTZ-induced seizures

Treatment	Dose (mg/kg)	Time to seizure onset (s)	% animals exhibiting convulsions	% animals surviving
Control	10	87.7 \pm 0.30	100	0
Diazepam	3	790.0 \pm 0.00*	33	100
EAS	100	400 \pm 0.78	50	83.33
	200	551.3 \pm 0.48	50	83.33
	300	652.9 \pm 10.3*	50	83.33

Values are expressed as the mean \pm SEM of six observations. * $p < 0.01$ versus saline treatment (One-way ANOVA followed by Dunnett's *post-hoc* test), PTZ: Pentylentetrazole, EAS: Extracts of *Alstonia scholaris*, *A. scholaris*: *Alstonia scholaris*, SEM: Standard error of mean

Table 2: Effects of *A. scholaris* extract on pilocarpine-induced seizures

Treatment	Dose (mg/kg)	Time to seizure onset (s)	% Animals exhibiting convulsions	% Animals surviving
Control	10	118.1 \pm 18.8	100	16.66
Diazepam	3	730.0 \pm 0.00*	00	100
EAS	100	373.9 \pm 18.7	50	83.33
	200	456.8 \pm 14.5*	50	83.33
	300	581.0 \pm 23.1*	50	83.33

Values are expressed as the mean \pm SEM of six observations. * $p < 0.01$ versus saline treatment (One-way ANOVA followed by Dunnett's *post-hoc* test), EAS: Extracts of *Alstonia scholaris*, *A. scholaris*: *Alstonia scholaris*, SEM: Standard error of mean

MES-induced seizures test

Treatment with EAS and diazepam significantly influenced MES-induced seizures in mice (Table 3). Dunnett's *post-hoc* test further revealed that *A. scholaris* (300 mg/kg, p.o.) significantly ($p < 0.01$) reduced duration of hind limb extension as compared to vehicle treated mice. These results were comparable with the effect of phenytoin (25 mg/kg, i.p.). In addition, EAS and phenytoin showed significant protection against mortality as well as seizures.

Phytochemical screening

Phytochemical screening of EAS indicated the presence of alkaloids, flavonoids, saponin, steroids, triterpenoids, phenol, and tannins.

DISCUSSION

The results revealed that treatment with EAS protected against PTZ, pilocarpine and MES - induced convulsions and mortality. In PTZ and pilocarpine -induced seizures, EAS at 100, 200, and 300 mg/kg, p.o. caused a significant increase in the latency to convulsions and also in the latency to death when compared with control, demonstrating a protective effect. It is well known that at high doses, the muscarinic agonist pilocarpine induces behavioral changes such as seizures and brain injury in rodents via cortical stimulation [15-17]. In contrast to pilocarpine, the convulsing mechanism of PTZ is poorly understood, but it is reported that this substance is able to inhibit chloride conductance by binding to sites of GABA_A receptor complex [18]. In MES induced seizure model, EAS decreased the duration of hind limb extension that was comparable to that of phenytoin. Phenytoin showed anticonvulsant effect by blocking the voltage-gated sodium channels [11].

The anticonvulsant activity of *A. scholaris* may be ascribed to the presence of alkaloids, flavonoids, and tannins, which have been found in its ethanolic extract by the phytochemical investigation. Previous studies have demonstrated that some alkaloids have anticonvulsant activity [19,20].

A. scholaris has various beneficial effects on the activities of the CNS, such as protective effect on stress and cognitive effect [9]; the anticonvulsant effect of this plant may be related to the central effect of its constituents. Consistent with this study, our results showed that EAS had anticonvulsant activity. Thus, further studies are needed to identify other constituents that may have effective anticonvulsant activities.

CONCLUSION

In conclusion, our data indicate that ethanolic EAS has anticonvulsant effects. This study provides a scientific rationale for the use of this ethanolic EAS for the amelioration of epilepsy observed in traditional medicine in India. However, *A. scholaris* contains multiple components such as alkaloids (ditamine, echitenine, and echitamine), saponin, steroids, triterpenoids, phenol, and tannins. Thus, more studies are necessary to clarify the antiepileptic components and the mechanisms underlying the properties.

Table 3: Effects of *A. scholaris* extract on MES induced seizures

Treatment	Dose (mg/kg)	Time to seizure onset (s)	% Animals exhibiting convulsions	% Protection against mortality
Control	10	11.83 \pm 1.47	100	16.66
Phenytoin	25	0.00 \pm 0.00*	00	100
EAS	100	5.88 \pm 0.94	50	83.33
	200	3.00 \pm 0.75*	50	83.33
	300	1.00 \pm 0.69*	00	100

Values are expressed as the mean \pm SEM of six observations. * $p < 0.01$ versus saline treatment (One-way ANOVA followed by Dunnett's *post-hoc* test), MES: Maximal electroshock, EAS: Extracts of *Alstonia scholaris*, *A. scholaris*: *Alstonia scholaris*, SEM: Standard error of mean

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