RESEARCH PAPER

Pharmacological Evaluation of *Alstonia scholaris* Fruit Extract for Antiepileptic Activity in Wistar Albino Rats.

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Abstract: Epilepsy is a brain disorder characterized by recurrent seizure due to irregular neuron behavior in brain. Though there are many antiepileptic medicines, the usage of these medicines causes some side effects along with resistance. In the past, *Alstonia scholaris* was a plant that was used for treating epilepsy and other neurological problems. However, no scientific study has been conducted on epilepsy yet now.
Aim: The aim of the current research was to evaluate the antiepileptic activity of *Alstonia scholaris* (EEAS) for antiepileptic activity in Wistar albino rats.
Material and Method: The Frutis of *Alstonia scholaris* were collected, authenticated and shade dried. The coarse powder of *Alstonia scholaris* was extracted from with ethanol using Soxhlet apparatus. The ethanolic extract of *Alstonia scholaris* fruit (EEAS) was used for the acute oral toxicity study according to OECD guideline 423. The antiepileptic activity of EEAS was evaluated in PTZ and PTX induced convulsion in Wistar albino rats.
Result: The acute toxicity study indicated that EEAS was safe up to 2000mg/kg,p.o. In both models, EEAS significantly increased the latency of convulsion and decreased the duration of conclusions in dose dependent manner when compared with the vehicle control group. In PTZ model, EEAS 200mg/kg, p.o significantly (**$p<0.005$) increased the latency of convulsion and highly significant (****$p<0.0001$) decreased the duration of convulsion when compared to vehicle control group. In PTX model, EEAS 200mg/kg, p.o significantly (*$p<0.5$) increased the latency of convulsion and highly significant (****$p<0.0001$) decreased the duration of convulsion when compared to vehicle control group. From the findings it was proved that the EEAS at 400 mg/kg,p.o exhibit the highly significant antiepileptic activity.
Conclusion: The present study revealed that ethanol extracts from the fruits of *Alstonia scholaris* had significant antiepileptic properties. This can be attributed to the participation of GABAergic and glutamatergic transmission pathways, among others. The results indicate that the use of the extract in treating epilepsy may be advantageous and needs further investigation.

Keywords: *Alstonia scholaris*, Epilepsy, Acute oral toxicity, Pentylenetetrazole, Picrotoxin, Sodium valproate.

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1. INTRODUCTION

Epilepsy is the most common neurological disorder characterized by recurrent spontaneous seizures in the brain. Approximately 50 million people worldwide are living with epilepsy. Epilepsy and an imbalance between excitation and inhibition, leading to seizures [1]. In addition to genetic and environmental factors, sequels of central nervous system infections, especially meningitis, viral encephalitis, cerebral malaria and neurocysticercosis, are the main causes of seizures and acquired epilepsy in the developing world [2]. Even though it was recognized as early as 2000 B.C, new concepts about its pathogenesis, etiology, and treatment are brought out almost every year. Abnormal synchronization of neuronal discharges is of recognized critical importance in seizures; however, the mechanisms underlying this pathological synchrony remain uncertain. In this context, there is growing interest in electrotonic communication via gap junctions, and speculation, based largely on studies in vitro and on ex vivo brain tissue that gap junctions may be important in the generation and propagation of seizures [3]. The pathogenesis of abnormal neuronal synchrony underlying seizures, formerly thought to be based mainly on the chemical synaptic transmission, now includes a role of gap junctional communication [4].

The most underlying mechanism in the development and progression of epilepsy and several other neurological disorders is oxidative stress. Oxidative

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stress is caused by excessive production of reactive oxygen species such as hydroxyl, superoxide anion radical, nitric oxide and hydrogen peroxide [5]. Several animal models of epilepsy are used to study the pathophysiology of the convulsive process and the antiepileptic activity of drugs in pre-clinical evaluation. Epilepsy is incurable but modern medicine can help control most cases of seizures but several conditions such as nonresponsive cases need to opt for surgery, neurostimulation or lifestyle changes [6]. In the past, the treatment of epilepsy was based on superstitious religious beliefs and ignorance. However, the present-day concept of the treatment of epilepsy is very much different from what it was earlier [7]. The WHO estimated that approximately 80% patients with epilepsy live in developing countries and most of them do not get adequate medical treatment. There are so many drugs available to treat epilepsy but none of them are free from side effects such as depression, hepatic failure ischemia, impaired cognition, motor disability, aplastic anemia etc. [8]. Despite the vast number of drugs introduced for the treatment of epilepsy, there is still a need for an ideal antiepileptic agent, with properties such as broad-spectrum activity, rapid onset of action, least side effects, good oral bioavailability, and low cost [9]. This situation can also be improved with the help of traditional and local medicines obtained from the natural flora of the region. A proper identification of medicinal properties and their scientific evaluation provide with much superior relief than the contemporary practice of medicine [10]. Medicinal plants have been used for centuries as remedies for human diseases, but their use has increased greatly over the past few decades [11]. India has a rich treasure of medicinal plants and has contributed to the development of the well-known system of Ayurveda, the science of life. Many medicinal plants from India have been shown to have activity by the traditional methods of psychoneuropharmacology [12].

Alstonia scholaris belongs to family Apocynaceae, commonly known as Blackboard tree, Indian devil tree and White cheese wood in English; Saptparna in Hindi and Vishwamukha in Sanskrit. It is well known remedy for the treatment of various types of disorders in the ayurvedic, homoeopathic [13]. *Alstonia scholaris* is widely distributed in southwestern China, India, Thailand, Malaysia, Philippines, Africa, Australia, etc. [14]. Different parts of *A. scholaris viz;* leaves, follicles and latex, a milky white secretion from follicles, are used in the treatment of various types of disorders in different medicinal systems. In alternative medicinal systems it is effective against different ailments such as asthma, malaria, fever, dysentery, diarrhea, epilepsy, skin diseases and snakebite. Latex is useful in application in ulcers, sores, tumors and rheumatoid pain. Fruits are useful in syphilis and epilepsy and used as tonic, anti-periodic and anthelmintic [15]. There have been various types of pharmacological activities reported on various part of *Alstonia scholaris* except the antiepileptic activity. Hence this activity is unexplored still now on this plant. To fulfill this gap, we must decide to evaluate the antiepileptic activity of ethanolic extract of *Alstonia scholaris* using PTZ and PTX induced seizure models in Wistar albino rats [16].

2. MATERIAL AND METHODS

2.1. Chemicals and reagent

Every chemical and reagent used in this study was of standard quality. The following chemicals and reagents were used throughout the study: Petroleum ether, Ethanol, Chloroform, acetic acid, boric acid, ethyl acetate, sulphuric acid, normal saline.

2.2. Equipments and glassware

Soxhlet apparatus, Mixer grinder, Rota evaporator, Digital balance, Beaker, Measuring cylinder, conical flask, funnel, Hot air oven.

2.3. Drugs and Inducing agent

Sodium valproate-Sun Pharma Laboratories Ltd. Mumbai), Pentylenetetrazol and Picrotoxin (SRL Pvt. Ltd. Mumbai), Normal saline (Bioy Labs Pvt. Ltd. Ropar).

2.4. Drug solution

Before being used, each chemical was freshly made in a standard saline solution (0.9%). Intraperitoneal (i.p.) injections of the solutions were given in a volume of no more than 10 mL/kg of body weight.

2.5. Approval of experimental animal:

Both sex of Wistar albino rats (2-3month), 150-200gm were used to perform the study. The experimental animals were housed in controlled conditions with a natural day-night cycle of 12 hours of darkness and 12 hours of light, at room temperature (22–24°C) and 50–60% air humidity. The perfect nutrition and clean drinking water were given to the animals. Prior to the start of the investigation, experimental animals were housed in polyethylene plastic cages in an ideal habitat for acclimatization for seven days. All the experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) of METGI Faculty of Pharmacy Moradabad and Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA). The protocol number the current was IAEC/02/2022/METFOP. All the protocols were conducted

2.6. Collection, identification and authentication of plant material

The fruit material of Alstonia scholaris was collected from the local area of Moradabad, Uttar Pradesh, India in the fruiting season in May. After collection the plant material was authenticated by Dr. Sunita Garg, head of RHMD at CSIR-NIScPR, New Delhi. A voucher specimen was deposited in the herbarium for future reference. The organization provides a voucher specimen no. NIScPR/RHMDConsult/2022/4125-56.

2.7. Dry and pulverization of fruit material

The fruit was washed twice with distilled water to get rid of dirt and foreign particles. The fruits were shade dried at room temperature till they became completely dry (10-15 days). The dried fruits were then coarsely

ground in a mechanical grinder and stored in an airtight container for more studies on the extraction.

2.8. Preparation of Extract

Alstonia scholaris fruit material (200g) was initially taken using 1000 petroleum ether (60–800) in a Soxhlet extractor for 24 hours to remove the fat and coloring substance. Following complete drying, the marc was extracted using 1000 ml of 95% v/v ethanol at 40–50°C for approximately 72 hours using the Soxhlet apparatus. In order to get crude dry extract at 40–50°C, the solvent was later evaporated using a rotatory vacuum evaporator. The final dried extracts were gathered in a container and refrigerated at 40C for additional research and characterization in the study after the percentage value of ethanolic extracts of fruits of *Alstonia scholaris* was calculated.

2.9. Approval of Animals and research protocols.

Wistar albino rats either sex, 2-2.5 months age and 150-200gm weight, were utilized in this study. Experimental animals were maintained under regulated conditions at room temperature (22-24°C) and 50-60% relative humidity, following a natural day-night cycle (12 hours dark/12 hours light). Animals were supplied with optimal food and distilled drinking water. Prior to starting the investigation, experimental animals were maintained in an appropriate laboratory setting for acclimatization for a duration of seven days. All experimental procedures or methods employed in the study received approval from the CPCSEA and IAEC of METGI Faculty of Pharmacy Moradabad. The IAEC registration number is 1867/PO/Re/S/16/CPCSEA, and the proposal approval number is METFOP/IAEC/02/2022.

2.10. Acute oral toxicity study

The acute oral toxicity study of Ethanolic extract of *Alstonia scholaris* (EEAS) was performed as per the OECD guideline no. 423. The three healthy, non-pregnant female Wistar albino rats (150-200gm) were selected for the acute oral toxicity study for each step. They were fasted overnight and weighed. The test dose of EEAS was prepared in distilled water and calculated according to the body weight of each animal. The calculated dose of EEAS was administered to each set of animals at a dose of 5,50, 300 and 2000mg/kg using oral cannula. Following dosage, the animals were monitored on an individual basis for the first 24 hours, paying particular attention to the first 4 hours, for behavioral changes and general toxicity indicators. After that, daily monitoring was maintained for a total of 14 days. The lower and higher doses of EEAS were selected based on the 2000mg/kg, p.o accompanied by $1/10^{th}$ and $1/5^{th}$ for the antiepileptic activity [17].

2.11. Evaluation of Antiepileptic activity

2.11.1. Pentylenetetrazol (PTZ) induced convulsion model

To investigate antiepileptic activity, a total of twenty-four Wistar albino rats were used. Four groups of experimental animals were assigned at random. There were six animals in each group. The animals receive all-drug treatments once a day for seven days. Animals of group I received distilled water 10ml/kg, p.o, Group II: received sodium valproate (200mg/kg, p.o); Group III: received EEAS (200mg/kg, p.o) and Group IV: received (400mg/kg, p.o.). The pentylenetetrazol at a dose of 80mg/kg, i.p used to induce convulsions in animals. The vehicle and EEAS treatment were given 30 minutes before PTZ injection. However standard drug sodium valproate 200mg/kg,p.o 15 minutes before PTZ injection. Animals were kept in their respective cages next for 30 min. The animals were observed for latency of convulsion and duration of convulsion [18].

2.11.2. Picrotoxin (PTX) induced convulsion model

Picrotoxin (PTX) causes convulsions through the inhibition of the GABA receptor/chloride channel and is the most used chemo convulsant in studies into the effects of antiepileptic drugs. To investigate antiepileptic activity, a total of twenty-four Wistar albino rats were used. Four groups of experimental animals were assigned at random. There were six animals in each group. The animals receive all-drug treatments once a day for seven days. Animals of group I received distilled water 10ml/kg, p.o; Group II: received sodium valproate (200mg/kg, p.o); Group III: received EEAS (200mg/kg, p.o) and Group IV: received (400mg/kg, p.o.). The picrotoxin at a dose of 7.5mg/kg, i.p used to induce convulsions in animals. The vehicle and EEAS treatment were given 30 minutes before PTZ injection. However standard drug sodium valproate 200mg/kg, p.o 15 minutes before PTZ injection. Animals were kept in their respective cages next for 30 min. The animals were observed for latency of convulsion and duration of convulsion [19].

2.11.3. Statistical Analysis

The results obtained from the study were expressed as mean ± SEM and data was analyzed using ANOVA by the GraphPad prism. All the treatment groups were compared with vehicle control group.

3. RESULT

3.1. Acute Toxicity study

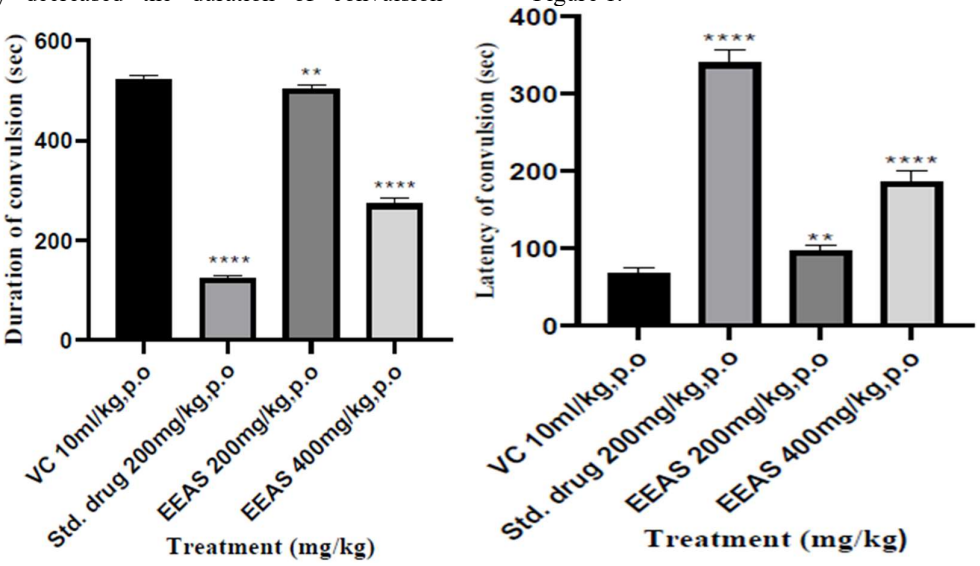
The acute oral toxicity study of EEAS was conducted according to the OECD guideline no. 423 at a limit dose 2000mg/kg, p.o. The plant extract EEAS was administered to the non-pregnant female Wistar albino rats up to 2000mg/kg by oral route using oral cannula. At the end of the toxicity study, it was observed that all the animals were safe at 2000mg/kg,p.o over a period of 14 days. They did not produce any sign of mortality and morbidity. Hence the drug EEAS was safe for the further evaluation of antiepileptic activity.

3.2. Antiepileptic activity

3.2.1. Effect of EEAS in pentylenetetrazol (PTZ) induced convulsion

The result of the present study demonstrated that the ethanolic extract of *Alstonia scholaris* (EEAS) possessed the antiepileptic activity in dose-dependent manner in PTZ induced convulsion when compared

with the vehicle control group. The animals treated with EEAS 200mg/kg, p.o significantly increased the latency of convulsion (**p<0.005), while EEAS at 400mg/kg, p.o extremely increased in latency of convulsion (****p<0.0001). Likewise, for the duration of convulsion study, EEAS at 200mg/kg, p.o has significantly decreased the duration of convulsion (**p<0.005), but EEAS at 400mg/kg, p.o extremely decreased the duration of convulsions (****p<0.0001). Also, the standard drug sodium valproate at 200mg/kg, p.o extremely decreased the duration of convulsion (****p<0.0001) when compared with the vehicle control group. The results of the study are illustrated in Figure 1.



(a) **(b)**

Figure 1. Effect of ethanolic extract of *Alstonia scholaris* (EEAS) on; **(a):** latency of convulsion and **(b):** duration of convulsion in PTZ induced convulsions in experimental convulsions in experimental animals. The data are expressed as Mean ± SEM (n=6), using one way ANOVA followed by appropriate post hoc test *p<0.05, **p<0.005, ***p<0.001 and ****p<0.0001 as compared with the vehicle control group.

3.2.2. Effect of EEAS in picrotoxin (PTX) induced convulsion

The result of the present study demonstrated that the ethanolic extract of *Alstonia scholaris* (EEAS) possessed the antiepileptic activity in dose-dependent manner in PTZ induced convulsion when compared with the vehicle control group. The animals treated with EEAS 200mg/kg, p.o significantly increased the latency of convulsion (*p<0.05), while EEAS at 400mg/kg, p.o extremely increased in latency of convulsion (****p<0.0001). Likewise, for the duration of convulsion study, EEAS at 200mg/kg, p.o has significantly decreased the duration of convulsion (**p<0.005), but EEAS at 400mg/kg, p.o extremely decreased the duration of convulsion (****p<0.0001). Also, the standard drug sodium valproate at 200mg/kg, p.o extremely decreased the duration of convulsion(****p<0.0001) when compared with the vehicle control group. The results of the study are illustrated in Figure 2.

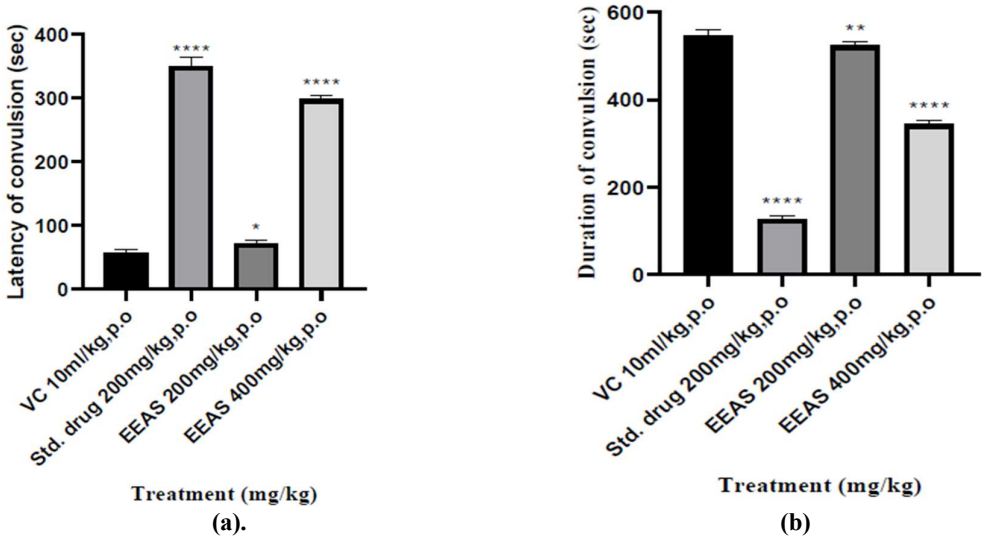


Figure 2: Effect of ethanolic extract of *Alstonia scholaris* (EEAS) on: **(a):** latency of convulsion and **(b):** duration of convulsion in PTZ induced convulsions in experimental convulsions in experimental animals. The data are expressed as Mean ± SD (n=6), using one way ANOVA followed by appropriate post hoc test *p<0.05, **p<0.005, ***p<0.0001 and ****p<0.0001 as compared with the vehicle control group.

4.. DISCUSSION

Epilepsy is a chronic neurological disorder that produces recurring convulsive or non-convulsive seizures affecting a variety of mental and physical functions. The increase in the number of patients with drug resistant seizure is reaching a sizeable population. Moreover, antiepileptic drugs show undesired CNS effects such as decreased cognitive abilities and psychiatric complications. Therefore, there is an obligation to explore effective and safe newer anticonvulsant agents with less toxicities from natural sources such as plants [20]. Pharmacological evaluation of medicinal plants has recently witnessed a growing interest amongst researchers worldwide. Research on the therapeutic potential of plants has surged over the years, with volumes of scientifically documented information showing considerable potential for medicinal plants to be used in the treatment of several diseases [21].

Alstonia scholaris, a species of the Apocyanaceae family, has been widely studied for its numerous pharmacological properties. It is native to the Indian subcontinent and Southeast Asia. *Alstonia scholaris* has been investigated for its anti-inflammatory, analgesic, antidiabetic, antihyperlipidemic, anti-malarial, and anti-microbial effects, in addition to it therapeutic potential against other diseases [22]. This study explores the anticonvulsant activity of ethanolic extract of *Alstonia scholaris* (EEAS) in Wistar albino rats in PTZ and PTX -induced convulsion. Preclinical studies have demonstrated that clinically effective anticonvulsants (Sodium valproate) reduce the severity of PTZ and PTX -induced seizures. As we know, this study is the first one about the antiepileptic effect of this plant's extract in biomedical literature. Acute toxicity test obtained in this study for fruits ethanolic extract of *Alstonia scholaris* (EEAS) proposed the extract of this plant is safe in, and/or non-toxic to rats. To investigate the possible interaction between GABAergic system and antiepileptic activity EEAS, sodium valproate was used.

PTX is another chemo convulsant agent which has been widely used in animal convulsion paradigms. It is also a noncompetitive GABA-A receptor antagonist for the GABA agonistic action on the receptor's chloride channel, acting as a channel blocker or allosteric modulator of the channel, which is thought to cause PTX's convulsive effect [23]. n our study, it was observed that ethanolic extract of *Alstonia scholaris* (EEAS) at 200 and 400mg/kg, p.o increased the latency of convulsion, shortened the duration of convulsion when compared with vehicle control group.

In the PTX induced convulsion model, it was observed that ethanolic extract of *Alstonia scholaris* (EEAS) at 200 and 400mg/kg, p.o increased the latency of convulsion, shortened the duration of convulsion when compared with vehicle.

The GABA-A channel is subject to regulation at several different binding sites. It contains two binding sites for GABA, which are also binding sites for the GABA agonists muscimol, gaboxadol, and bicuculline, an allosteric binding site for benzodiazepines that bind benzodiazepine agonists causing channel closing and benzodiazepine inverse agonists causing channel opening, as well as competitive benzodiazepine antagonists which prevent the actions of both the benzodiazepine agonists and inverse agonists. Furthermore, the GABAA receptor contains allosteric binding sites for several other compounds, which include PTZ and PTX [24]. The exact binding modes for the latter are not fully known and are presumably located spatially different from each other and involved in different modes of modulation of the receptor channel. It is known that PTX binding itself is

allosterically regulated in the presence of muscimol, bicuculline, and benzodiazepine agonist and inverse agonists and combinations thereof in complex mode. The effectiveness of extracts in above two models is suggestive that they may either be modulating the GABAergic or glutamatergic activity and further studies are warranted to establish this modulatory effect [25].

5. CONCLUSION

The study reveals that ethanolic extract of *Alstonia scholaris* (EEAS) shows dependent anti-convulsant activity, The extracts helped in reversing these symptoms suggestive of probable modulation of glutamatergic neurotransmission. Moreover, it provides pharmacological evidence for the use of *Alstonia scholaris* in traditional system of medicine for the treatment of epilepsy. However, further there is need to isolate and characterize the bioactive compound that supports and the molecular mechanism of *Alstonia scholaris* in neuroprotection.

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Conflict of Interest

No conflict of interest declared by the authors.

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