

Pharmacological Role of *Alstonia scholaris* Leaves for its Anticonvulsant and Sedative Action

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ABSTRACT

Background & Objectives: *Alstonia scholaris* leaves were used to evaluate the anticonvulsant and sedative potential on the basis of its traditional and folklore uses in epilepsy as containing chemical constituents like alkaloids, saponins, terpenoids and flavonoids, which show the CNS activity. On the basis of its folk use, the study was designed to evaluate the anticonvulsant and sedative potential of ethanolic extract of *Alstonia scholaris* (EEAS) leaves.

Methods: *For anticonvulsant study*-MES induced convulsion, Isoniazid induced convulsion and pentylenetetrazole (PTZ) induced convulsion methods were used. *For sedative study*-Locomotor activity of mice using actophotometer and pentobarbitone induced sleeping time model in mice were performed.

Results and discussion: The extract is effective in Isoniazid, PTZ and Maximal Electroshock (MES) induced convulsion model. 400mg/kg dose of the EEAS shows the maximum protection of epilepsy induced by the MES and the chemical convulsant as compared to low dose (200 mg/kg) of *Alstonia scholaris*. EEAS also possess the sedative activity when tested in Pentobarbitone induced sleep, further it decreases the locomotor activity in mice. The 400mg/kg extract potentiate the effect of Pentobarbitone.

Conclusion: The study concluded that the ethanolic extract of *Alstonia scholaris* possesses antiepileptic and sedative potential. These effects may be due to alteration in the GABA mediated chloride channel of neurons associated with sleep activity.

Keywords: *Alstonia scholaris*, Anticonvulsant activity, Sedative activity, Maximal electroshock, Isoniazid, Pentylenetetrazole, Pentobarbitone.

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INTRODUCTION

Epilepsy is the second most common neurological disorder in India¹. Moreover, approximately 30% of people with epilepsy have “intractable seizures” that do not respond to even the best available treatment^{2,3}. Right now accessible antiepileptic therapy consisting about 30% of patient with regular seizures in their life. This fact demands new safer antiepileptic drugs for better and effective control of epilepsy^{4,5}. There are a number of synthetic antiepileptic drugs currently available for the management, control and/or treatment of epilepsy. But most of these synthetic drugs are not only inaccessible and unaffordable and they also possess many toxic adverse effects⁶. Currently used antiepileptic drugs cause side effects which vary in severity from minimal impairment of the central nervous system to aplastic anaemia or hepatic failure and teratogenicity⁷.

In developing countries up to 80% of the population depend on traditional systems of medicines or folk remedies for their primary health care requirements^{8,9}. Moreover, plentiful herbal medicines are active on the central nervous system (CNS), and have shown the potential efficacy on chronic conditions such as anxiety, depression, headache, seizures which does not respond well in the direction of conventional treatments^{10,11}.

Alstonia scholaris a tree belonging to the family Apocynaceae has been used since time immemorial in the folklore and traditional systems of medicine in India¹². *Alstonia scholaris*, leaves are used to evaluate the anticonvulsant and sedative activity on the basis of its traditional uses on central nervous system and also depend on its chemical constituents that are already proven for CNS properties^{13,14}.

MATERIALS AND METHODS

Plant Material

The leaves of *Alstonia scholaris* (family-Apocyanaceae) were collected in the month of October 2012 from Forest research institute Dehradun. The plant material was identified by Mr. Gaurav Upadhyay Assistant Professor, Siddhartha Institute of Pharmacy, Dehradun.

Preparation of ethanolic extract

About a kilogram of fresh leaves of *Alstonia scholaris* were collected from Forest Research Institute, Dehradun (Uttarakhand). The leaves were first washed free of sands and debris. Wash water was blotted off then the leaves were dried in shade and ground to powder. A quantity of the powder (500g) was weighed and soxhlet extracted with ethanol (95%) for 18 hrs. The extract was concentrated to a small volume by the rotary evaporator apparatus and then dried at room temperature. The extract obtained was in the form of thick paste and Dark green in color. The yield of ethanol extract is 42gm^{15,16}.

Preparation of dose

Weighed quantity of ethanolic extract of *Alstonia scholaris* (EEAS) were suspended in distilled water using 1% Tween 80 and administered orally to experimental animals. Suspension of extract was prepared freshly. The extracts were administered at a constant volume of 10ml/kg for each animal¹⁷.

Experimental animals

Swiss albino mice (20–25g) and Wistar albino rats (150–200g) of either sex were used for the study. The inbred colonies of mice and rats were obtained and maintained by Siddhartha Institute of Pharmacy, Dehradun, India for experimental purpose. The animals were maintained under controlled conditions of temperature

(23±2°C), humidity (50 ± 5%) and 12 h light–dark cycle (light on from 06:00 to 18:00 h). All the animals were acclimatized for seven days before the study. They had free access to standard pellets as basal diet and water ad libitum. Further, to minimize the chances of non-specific stress animals were habituated to laboratory conditions for 48 h prior to experimental protocol. All the studies conducted were approved by the Institutional Animal Ethical Committee Siddhartha Institute of Pharmacy, Dehradun, India (1435/PO/A/11/CPCSEA).

Drugs

Isoniazid, Phenytoin, PTZ, Diazepam, Pentobarbital sodium, Tween 80 and Ethanol were purchased from Himgiri traders, 121-kanwali Road, Dehradun-248001 (India). All the chemicals used in the study were of analytical grade.

PRELIMINARY PHYTOCHEMICAL SCREENING

The powdered leaves of *Alstonia scholaris* was subjected to preliminary phytochemical analysis to test for the presence of phytochemical constituents using the following methods:

Tests for Carbohydrates

Molisch's test- sufficient quantity of the extract was supplied to a few drops of α -naphthol solution in alcohol, shaken and concentrated H_2SO_4 was added from the sides of the test tube. It was observed for a violet ring at the junction of two liquids.

For Reducing Sugars

a) Fehling's test

1ml Fehling's A and 1ml Fehling's B solutions were mixed and boiled for one min. Sufficient quantity of EEAS was added. Heated in boiling water bath for 5-10 min and

observed for a yellow, then brick red precipitate.

b) Benedict's test

Benedict's reagent and test sample were mixed in a test tube. Heated in boiling water bath for 5 min. The solution was observed for green, yellow or red depending on amount of reducing sugar present in it.

Test for Mono-saccharide's

a) Barfoed's test

Barfoed's reagent and EEAS extract were mixed. Heated for 1-2 min, in boiling water bath and cooled. Observed in red color formation.

Tests for Proteins

a) Biuret test (General test)

Sufficient quantities of EEAS were added at 4% NaOH and a few drops of 1% $CuSO_4$ solution and observed for violet or pink color formation.

b) Million's test (for proteins)

Mixed Sufficient quantity of the EEAS with 5ml Million's reagent, the white color precipitate was obtained. The precipitate was warmed, which turns brick red.

Tests for Steroids

a) Salkowski Reaction

Sufficient quantity of EEAS, 2ml chloroform and 2ml concentrated H_2SO_4 was added. Shook well, chloroform layer appeared red and acid layer showed greenish yellow fluorescence on standing.

b) Liebermann-Burchard Reaction

Mixed sufficient quantity of the EEAS with chloroform. Added 1-2ml glacial acetic acid and 2 drops concentrated H_2SO_4 from the side of the test tube, and

observations are carried out firstly for red, followed by blue and ultimately green.

Tests for Amino Acids

a) Ninhydrin test (General test)

Sufficient quantity of EEAS and 3 drops 5% Ninhydrin solution was heated in boiling water bath for 10 min and observed for purple or bluish color.

b) Test for Tyrosine

Sufficient quantity of EEAS and 3 drops Million's reagent was heated in test tubes. The solution was observed for dark red color.

Tests for Flavonoids

a) Shinoda test

To dried powder or extract, add 5ml 95% ethanol, few drops of concentrated HCl and 0.5 gm magnesium turnings. Pink color was observed.

b) To the small quantity of residue, added the lead acetate solution observed for yellow colored precipitate.

c) The addition of the increasing amount of sodium hydroxide of the residue was observed as to whether it showed yellow coloration, which was decolorised after addition of acid¹⁸.

d) Ferric chloride test

To test solution, added a few drops of ferric chloride solution observed for intense green color.

Tests for Alkaloids

a) Dragendorff's test

Sufficient quantity of the EEAS added a few drops of Dragendorff's reagent and was observed to orange brown precipitate.

b) Mayer's test

For Sufficient quantity of EEAS, few drops of Mayer's reagent were added & observed to precipitate.

c) Hager's test

Sufficient quantity of EEAS with Hagers reagent was observed for yellow precipitate.

d) Wagner's test

Sufficient quantity of the EEAS with a few drops of Wagner's reagent was observed for reddish brown precipitate.

Tests for Tannins and Phenolic Compounds

Sufficient quantity of EEAS, added a few drops of the following solutions and was looking for respective coloration or precipitate:

a) 5% Ferric chloride solution

Intense bluish-black color.

b) Lead acetate solution

White precipitation.

c) Bromine water

Decoloration of bromine water.

d) Acetic acid solution

Red color solution.

Tests for Glycosides

a) Baljet's test

By using sodium picrate solution and observing yellow to orange color.

b) Legal's test (For cardenoloids)

To aqueous or alcoholic test solution, added 1ml pyridine and 1ml sodium nitroprusside, observed for pink to red color.

c) KellarKillani test

To 2ml extract added glacial acetic acid, one drop of 5% FeCl₃ and concentrated H₂SO₄, observed for reddish brown color at the junction of the two liquid and upper layers bluish green.

Tests for Saponin Glycosides

Foam test

The drug extract or dry powder was shaken vigorously with water. Persistent foam was observed.

ANTICONVULSANT ACTIVITY

Maximal electroshock induced convulsions

The animals were divided into four groups (n=6). The group I animals served as control receiving normal saline p.o., Group II served as drug control receiving Phenytoin 25mg/kg, i.p. and Group III & IV animals were administered EEAS at doses of 200 and 400mg/kg, p.o. 60 min before application of electric shock. After 30 and 60 min, seizures were induced to all the groups of animals using electro-convulsometer (Bio Med Care). A 60 Hz alternating current of 150 mA intensity elicited MES seizures for 0.2 s in rats. A drop of 0.9% w/v NaCl (acting as electrolyte solution) with lignocaine was applied to the corneal electrodes proceeding for application in the rats. This increases the contact and reduces the incidence of fatalities. The observed duration of various phases like flexion, extensor, clonic convulsions, stupor, recovery/ death in epilepsy was tabulated.

Isoniazid induced convulsions

Rats were divided into four groups (n=6). The group I received vehicle + Isoniazid (300mg/kg, i.p.); Group II received diazepam + Isoniazid; Group III received EEAS 200 + Isoniazid; Group IV received EEAS 400 + Isoniazid. The treatment of EEAS was administered one hour before the administration of Isoniazid and diazepam was

administered 30 min before the administration of Isoniazid. The rat was placed in an isolated perplex chamber, during the next 120 min the occurrence of clonic seizures, tonic seizures and death was recorded. The percentages of seizures or deaths occurring in the control group were taken as 100%. The suppression of these effects in the treated groups was calculated as percentage of controls.

Pentylentetrazole induced seizure

The animals were divided into four groups (n = 6) and Group I animals served as control receiving normal saline, p.o.+ PTZ 80mg/kg i.p. Group II served as drug control receiving diazepam 4mg/kg, i.p.+ PTZ 80mg/kg i.p. And Group III receives EEAS 200mg + PTZ 80mg/kg i.p. and IV animals were administered EEAS 400mg/kg, p.o. + PTZ 80mg/kg i.p. one hour before the administration of PTZ and diazepam 30 min before the administration of PTZ. The endpoint was characterized by onset of general clonus activity and observed for the duration of 2 hours.

SEDATIVE ACTIVITY

Locomotor activity

In this method, the movement of the animals was observed by the interruption of a beam of light falling on a photo cell, which was recorded and displayed digitally for locomoter activity. The animals were divided into four groups, each consisting of six animals. The first group received normal saline, p.o. and the second group was subjected to standard drug, diazepam 4 mg/kg, i.p. Further, third and fourth groups were administered EEAS (200mg/kg and 400 mg/kg, p.o.) respectively. The mice were placed in Actophotometer after 30 min of i.p. and 60 min of p.o. dose for observing the activity and the basal activity score was recorded after placing each mouse in Actophotometer for 10 min.

Pentobarbitone induced sleep

For this purpose the experimental animals of either sex were divided into 4 groups each having 6 mice. The first group received normal saline, p.o. and the second group was subjected to standard drug, diazepam 4mg/kg, i.p. Further, third and fourth groups were administered EEAS (200mg/kg and 400mg/kg, p.o.) respectively. After 30 min of i.p. and 60 min of p.o. dosing, Pentobarbital sodium (40mg/kg, i.p.) was administered to all the mice. Each mouse was observed for the loss of righting reflex, during which the mice cannot roll back when turned over. The time interval between the loss and the recovery of righting reflex was recorded for the indexing of hypnotic effect.

Statistical analysis

The recorded data was expressed as Mean \pm S.E.M. and the significance values shows as $a = *p < 0.05$, $b = **p < 0.01$, $c = ***p < 0.001$. Further the statistical comparisons were performed by one-way ANOVA followed by Tukey's post-test using Graph Pad Prism version 5.01, USA.

RESULTS

Phytochemical investigation

Phytochemical investigation of ethanolic extract revealed the presence of phytochemical constituents such as; Carbohydrates, Reducing sugars, Saponins, Alkaloids, Tannins/phenolic compounds, Glycosides, Terpenoids, Flavonoids and absence of Steroids only.

ASSESSMENT OF ANTICONVULSANT ACTIVITY

Maximal electroshock induced convulsions

Administration of maximal electroshock (MES) through the corneal electrode to vehicle treated animals produced different phases of convulsion such as flexion, hind limb extensor, clonus, stupor and

recovery. Treatment with EEAS at a dose of 200mg/kg produced significant reduction in the hind limb extensor phase of convulsion as compared to vehicle treated animals. The higher dose of EEAS (400mg/kg) resulted in reduced flexion, hind limb extension and stupor phase of convulsion in comparison to vehicle treated group (fig. 1). Standard antiepileptic drug phenytoin (25mg/kg) completely blocked the hind limb extensor phase and produced significant reduction in the stupor phase (table 6).

Isoniazid induced convulsions

Pre-treatment of animals with EEAS in the dose of 200mg/kg delayed the onset of convulsion as compared to vehicle treated group; this effect was lower as compared to higher doses of the EEAS (400mg/kg). Standard antiepileptic drug diazepam (4 mg/kg) significantly delayed the onset of convulsion (table7).

In vehicle treated group, INH induced convulsion in all 6 rats, pre-treatment of animals with 200mg/kg of the EEAS did not block the convulsion in any rat whereas in higher dose 400mg/kg of EEAS block the convulsion in 2 rats out of 6 rats. Standard antiepileptic drug diazepam (4mg/kg) blocks the convulsion in 3 rats out of 6 rats (table7).

PTZ induced convulsions

Pre-treatment of animals with EEAS at a dose of 200mg/kg delayed the onset of convulsion and significantly decreases the duration of convulsion as compared to vehicle treated group; this effect was lesser as compared to higher doses of the EEAS (400mg/kg). Standard antiepileptic drug diazepam (4mg/kg) was completely blocking the convulsions. Further, in vehicle treated group, PTZ induces convulsion in all 6 rats, pre-treatment of animals with 200mg/kg of EEAS shows only 33% (2 rats) blocked of the convulsion, whereas at higher dose 400mg/kg

of EEAS block the convulsion about 67% (4 rats) out of 6 rats (table 8).

ASSESSMENT OF SEDATIVE ACTIVITY

Effect of EEAS on Locomotors activity

Pre-treatment group of animals administered with different dose of EEAS (200mg/kg and 400mg/kg) revealed statistically significant ($p < 0.01$ and $p < 0.001$) reduction in locomotors activity when compared to the vehicle treated group (table 9). Furthermore the diazepam treated group also revealed statistically significant ($P < 0.001$) decline in locomotors activity.

Effect of EEAS on Pentobarbital induced sleeping time

In case of pre-treatment group with EEAS dosing of 200mg/kg, there was no significant effect on onset of sleeping time observed. Whereas the same dose offered significant ($p < 0.05$) increase in duration of sleeping time when compared to vehicle treated group. On the other hand at higher doses of the EEAS (400mg/kg) there was significantly decrease in onset of sleeping time ($p < 0.001$) and increase in duration of sleep when compared to vehicle treated group (table 10). Further the standard antiepileptic drug, diazepam (4mg/kg) significantly ($p < 0.001$) depicted decrease in the onset of sleeping time while increase in the duration of sleep.

DISCUSSION

The present study have been designed to evaluate the anti-epileptic and sedative effects of ethanolic extract of the leaves of *Alstonia scholaris* using different animal models as; electroshock induced model & chemically induced model for epilepsy, whereas Pentobarbitone was used to evaluate sleeping time, while Actophotometer for sedative activity. The observations emanated

in the present study indicated that ethanolic extract of *Alstonia scholaris* leaves possessed anticonvulsant activity against seizures induced by maximal electroshock, Pentylenetetrazole (PTZ) and Isoniazid. It also possessed sedative activity on Pentobarbitone induced sleeping model. Further it decreased the locomotor activity in mice¹⁹. On the other hand, ethanolic extract of *Alstonia scholaris* leaves significantly ($p < 0.001$) decreased the extension phase and possessed the anticonvulsant activity in a dose dependent manner in rats. While the EEAS at higher dose (400mg/kg p.o.) revealed the maximum protection as compared to the standard drug i.e. Phenytoin at a dose of 25mg/kg i.p.

The convulsions induced by Isoniazid (INH) involve disruption of GABA ergic neurotransmission in the central nervous system, leading to deficiency of pyridoxine (vitamin B6), by inhibition of pyridoxine phosphokinase, the enzyme that converts pyridoxine to active B6, which is required by glutamic acid decarboxylase to convert glutamic acid to GABA. Decreased levels of GABA are believed to potentiate seizures. The present study revealed that ethanolic extract of *Alstonia scholaris* leaves (EEAS) exhibited a significant anticonvulsant activity against Isoniazid (INH) induced seizures in rats. Highest anticonvulsant activity was observed at a dose of 400mg/kg p.o. with a significant ($p < 0.001$) increase in meantime of latency for the onset of seizure²⁰.

While in case of Pentylenetetrazole, it induced seizures in all groups of animals but the mechanism is uncertain. However, previous studies shown that the Pentylenetetrazole may produce seizures by inhibiting GABA ergic mechanisms²¹. Further the administration of EEAS at a dose of 400mg/kg p.o. shown the significant ($p < 0.001$) anticonvulsant effect in PTZ induced model and also decrease in the number of death as compared to control

group. The standard antiepileptic drugs like diazepam (4 mg/kg.i.p) completely block the convulsion and produce 100% protection by enhancing GABA-mediated inhibition in the brain. The study suggests that, the anticonvulsant effects exposed by the standard drugs against Pentylenetetrazole-induced seizures might be due to the activation of GABA neurotransmission. Since EEAS similarly antagonized seizures elicited by Pentylenetetrazole therefore, it is evident that it may also be exerting its anticonvulsant effects by affecting GABAergic mechanism²².

The preliminary phytochemical investigations of *A. scholaris* leaf extract revealed the presence of carbohydrates, alkaloids, saponins and reducing sugars. The sedative effects of the EEAS might therefore be due to any one or combination of these phytochemicals. The plant extract was employed in a pentobarbital induced sleeping time study, in order to investigate, whether it exhibits sedative/hypnotic effects²³. It was observed the EEAS did not produce hypnosis up to doses of 400mg/kg p.o. in mice, however, the animals were found to be dull, calm and relaxed. Further the sedative effect of the plant extract was confirmed by the pre-treatment of the animals with the plant extract (400 mg/kg) which prolonged significant ($P<0.001$) pentobarbital induced sleeping time in mice from 60.33 ± 4.364 to 115.7 ± 4.49 min (mean \pm S.E.M. n=6), similar to that of diazepam (standard sedative drug) at the dose of 4mg/kg. While the EEAS decreased locomotor activity to a lesser extent than diazepam, offering a better potential for antiepileptic effects^{24,25}.

CONCLUSION

On the experimental basis it was concluded that, the chemical constituents present in ethanolic extract of the leaves of *Alstonia scholaris* have excellent antiepileptic and sedative potential. These

neuropharmacological properties are possibly mediated via facilitation of GABA transmission or via opening of chloride channels.

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Table 1. Experimental design: Effect of EEAS on MES induced convulsions in rats

Group	Treatment & Dose	Observation
I	Control (normal saline p.o.) + Electroshock (150 MA. For 0.2 sec.)	Tonic extension of the hind limbs
II	Phenytoin (25mg/kg, i.p.) + Electroshock (150 MA. For 0.2 sec.)	
III	EEAS (200 mg/kg, p.o.) + Electroshock (150 MA. For 0.2 sec.)	
IV	EEAS (400 mg/kg, p.o.) + Electroshock (150 MA. For 0.2 sec.)	

Table 2. Experimental design: Effect of EEAS on INH induced convulsions in rats

Group	Treatment & Dose	Observation
I	Control (normal saline, <i>p.o.</i>) + Isoniazid (300mg/kg <i>i.p.</i>)	Onset of convulsions
II	Diazepam (4 mg/kg, <i>i.p.</i>) + Isoniazid (300mg/kg <i>i.p.</i>)	
III	EEAS (200 mg/kg, <i>p.o.</i>) + Isoniazid (300mg/kg <i>i.p.</i>)	
IV	EEAS (400 mg/kg, <i>p.o.</i>) + Isoniazid (300mg/kg <i>i.p.</i>)	

Table 3. Experimental design: Effect of EEAS on PTZ induced convulsions in rats

Group	Treatment & Dose	Observation
I	Vehicle (normal saline, <i>p.o.</i>) + PTZ (80mg/kg <i>i.p.</i>)	Onset of convulsions
II	Diazepam (4 mg/kg, <i>i.p.</i>) + PTZ (80mg/kg <i>i.p.</i>)	
III	EEAS (200 mg/kg, <i>p.o.</i>) + PTZ (80mg/kg <i>i.p.</i>)	
IV	EEAS (400 mg/kg, <i>p.o.</i>) + PTZ (80mg/kg <i>i.p.</i>)	

Table 4. Experimental design: Locomotor activity of mice using Actophotometer in rats

Group	Treatment & Dose	Observation
I	Vehicle (normal saline <i>p.o.</i>)	Basal activity scores for 10 min. (Before and After)
II	Diazepam (4mg/kg <i>i.p.</i>)	
III	EEAS (200mg/kg <i>p.o.</i>)	
IV	EEAS (400mg/kg <i>p.o.</i>)	

Table 5. Experimental design: Pentobarbitone induced sleeping time in mice

Group	Treatment & Dose	Observation
I	Vehicle (normal saline <i>p.o.</i>) + Pentobarbital sodium 40mg/kg <i>i.p.</i>	Onset of sleep
II	Diazepam (4mg/kg <i>i.p.</i>) + Pentobarbital sodium 40mg/kg <i>i.p.</i>	
III	EEAS (200mg/kg <i>p.o.</i>) + Pentobarbital sodium 40mg/kg <i>i.p.</i>	
IV	EEAS (400mg/kg <i>p.o.</i>) + Pentobarbital sodium 40mg/kg <i>i.p.</i>	

Table 6. Effect of pre-treated EEAS on maximal electroshock induced convulsions in rats

Group	Treatment	Various Phases of Convulsions (in sec)			
		Flexion	Extension	Clonus	Stupor
I	Vehicle (1% tween 80, 1ml/kg p.o.)	09.33±0.76	07.66±0.71	14.17±0.98	13.33±0.61
II	Phenytoin (25mg/kg i.p.)	02.83±0.70 ^c	00±00	09.00±0.85 ^b	08.00±0.36 ^c
III	EEAS (200mg/kg p.o.)	06.83±0.65	05.50±0.34 ^a	12.33±0.42	12.83±0.54
IV	EEAS (400mg/kg p.o.)	04.50±0.7 ^b	02.50±0.50 ^c	10.00±0.36	10.17±0.47 ^b

Values are expressed in mean±SEM, whereas; a= *p<0.05, b= **p<0.01, c= ***p<0.001; compared with vehicle treated group. Statistical analysis done by one-way ANOVA followed by Tukey's test.

Table 7. Effects of pre-treated EEAS on Isoniazid induced convulsions in rats

Group	Treatment	Onset of convulsion (Min)	No of animals showing
I	1 % Tween 80 (1ml/kg p.o.)	49.17 ± 1.74	06
II	Diazepam (4mg/kg i.p.)	105.3± 6.94 ^c	03
III	EEAS (200mg/kg p.o.)	67.57±4.20 ^a	06
IV	EEAS (400mg/kg p.o.)	84.80±5.24 ^c	04

Values are expressed in mean±SEM, whereas; a= *p<0.05, c= ***p<0.001; compared with vehicle treated group. Statistical analysis done by one-way ANOVA followed by Tukey's test.

Table 8. Effects of pre-treated EEAS on PTZ induced convulsions in rats

Group	Treatment	No of animals showing	Onset of convulsion (Min)	Duration of convulsion	No of death	Protection (%)
I	1 % Tween 80 (1ml/kg p.o.)	06	52.67±5.96	54.17±2.96	06	0 %
II	Diazepam (4mg/kg i.p.)	00	00	00	00	100 %
III	EEAS (200mg/kg p.o.)	06	98.33±13.17 ^a	41.83±3.64 ^a	04	33 %
IV	EEAS (400mg/kg p.o.)	06	208.5±11.94 ^c	7.66±1.88 ^c	02	67 %

Values are expressed in mean±SEM, whereas; a= *p<0.05, c= ***p<0.001; compared with vehicle treated group. Statistical analysis done by one-way ANOVA followed by Tukey's test.

Table 9. Effect of EEAS on Locomotor activity in mice

Group	Treatment	Locomotor activity in 10 min		% change in activity
		Before	After	
I	Normal saline	199.8±24.28	206.3±11.38	7.84±4.77
II	Diazepam (4mg/kg, i.p.)	195.5±33.39	99.33±5.53 ^c	48.05±8.89 ^a
III	EEAS (200mg/kg.p.o.)	185.7±18.44	142.3±14.84 ^b	18.74±12.83
IV	EEAS (400mg/kg.p.o.)	201.2±7.19	115.3±3.67 ^c	32.75±9.22

Values are expressed in mean±SEM, whereas; a= *p<0.05, c= ***p<0.001; compared with vehicle treated group. Statistical analysis done by one-way ANOVA followed by Tukey's test.

Table 10. Effect of EEAS on Pentobarbital induced sleeping time

Group	Treatment	Onset of sleep (Min)	Duration of sleep(Min)
I	Normal saline	25.17±2.90	60.33±4.36
II	Diazepam (4mg/kg i.p.)	10.53± 6.94 ^c	125.3±3.81 ^c
III	EEAS (200mg/kg p.o.)	21.67±1.22	80.17±5.60 ^a
IV	EEAS (400mg/kg p.o.)	13.33±0.98 ^c	115.7±4.49 ^c

Values are expressed in mean±SEM, whereas; a= *p<0.05, c= ***p<0.001; compared with vehicle treated group. Statistical analysis done by one-way ANOVA followed by Tukey's test.

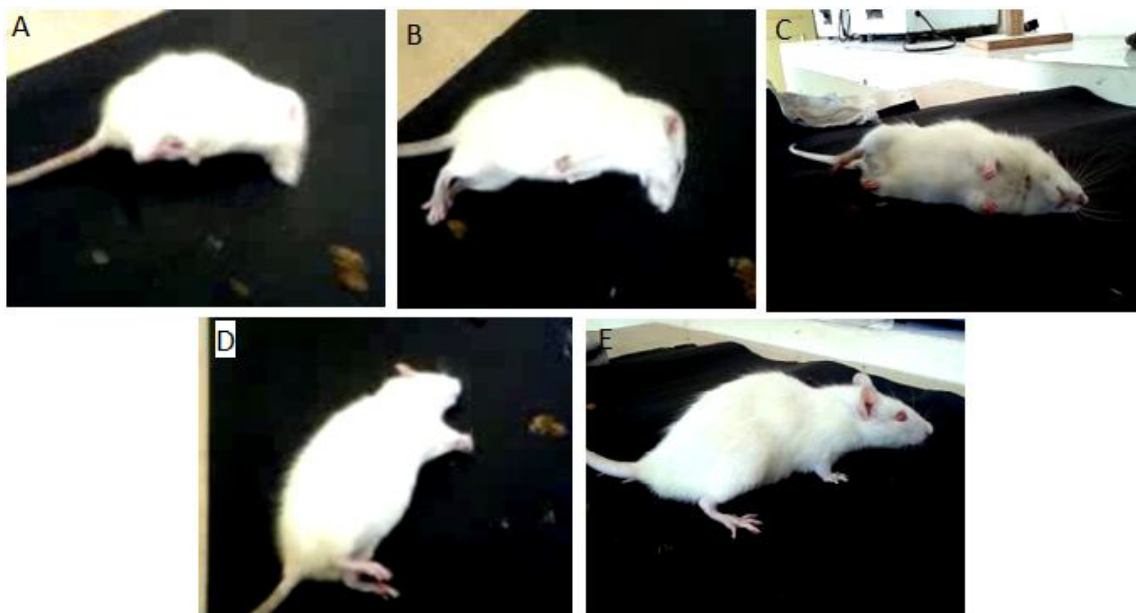


Fig.1. Different Phases of convulsions shown as (A) Flexion (B), Extension, (C) Tonic-clonic convulsion, (D) Stupor, (E) Recovery