Research Article

Anticonvulsant Assessment of Alcoholic and Aqueous Extracts of Leaves of *Shorea robusta*

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Received: 10-04-2019; **Revised:** 22-05-2019; **Accepted:** 05-06-2019.

ABSTRACT

The present study was undertaken to evaluate the CNS activity of different extracts of leaves of *Shorea robusta*. Swiss albino mice were subjected to dosages of 100, 300 & 500mg/kg each extract for the study for anticonvulsant action. The methanolic and aqueous extracts were evaluated using PTZ induced seizures (PTZ 100 mg/kg), picrotoxin induced seizures (picrotoxin 3.5 mg/kg), strychnine induced seizures (strychnine 4mg/kg), and MES (Maximum electroshock method, 75mA current). Diazepam and phenytoin were used as standard drug with the dose of 4 mg/kg and 25mg/kg respectively in all the experimental models. The significance of final result was calculated by applying one way ANOVA followed by Dunneet's t- Test and compared with the control group. The software used for calculating the significance was Graph Pad (All values expressed as mean ± SEM; n=6 mice in each group, significance values are *p<0.05 and ***p<0.001.) The methanol extracts showed significant anticonvulsant activity by increasing the time of latency and reducing duration of extensor phase of seizure in MES and PTZ induced seizures. However, picrotoxin and strychnine induced seizure model shows little or no significant results. The ideal dose from the methanol extract was 300 and 500 mg/kg.

Keywords: *Shorea robusta*, Epilepsy, MES, PTZ, Treatment Gap, ANOVA

INTRODUCTION

Epilepsy is a neurological disorder/disease marked by sudden recurrence episodes of sensory disturbance, loss of unconsciousness, or convulsions associated with abnormal electrical activity in brain. International league against epilepsy (ILAE) and International Bureau of epilepsy (IBE) has revised the definition and included the term disease instead of disorder as it is a heterogeneous condition [1], so is cancer and heart disease and those are called disease. Epilepsy affects about 50 million people worldwide and 10 million in India, according to World Health Organization (WHO). An epidemiological study suggested a prevalence of 6.8/1000 in USA and 1% in India. About 80% of total epileptic patients reside in a developing country which shows that the prognosis of epilepsy is more in developing countries compared to developed countries [2]. This may be due to higher incidence of antecedent factor such as brain infection, cranial and prenatal trauma and parasitic infection.

Shorvon and Former in 1988 indicated that ≥ 80% patients with epilepsy in developing countries do not receive medication [3]. N. E. Bharucha reported the treatment gap between epileptic patients and those who receive appropriate antiepileptic drugs (AEDs), which is about 54% to 57%. Although there are many antiepileptic drugs are available in market even then due to this treatment gap many patients 20-30% do not receive proper medication. However have type of epilepsy that is not managed by present AEDs. This urges the requirement of more antiepileptic agents which are efficacious and safe[4].

The present research work is an attempt to minimize this treatment gap and to improve quality of life of epileptic patients. *Shorea robusta* (Dipterocarpaceae) is a tree commonly known as sal or shala tree, belonging to the family Dipterocarpaceae. It is already reported for its analgesic, antipyretic, anti-inflammatory, and anti-obesity activity [5] which suggests that this plant may have some CNS activity too. In addition to the Ayurvedic system of medicine, this tree is widely used in Unani medicine. *Shorea robusta* is a large, deciduous tree up to 50 m tall and with a dbh (diameter at breast height) of 5 m; these are exceptional sizes, and under normal conditions *S. robusta* trees attain a height of about 18-32 m. It is natural to the temperate region of Himachal Pradesh, Uttranchal and some other regions of north India [6-7].

MATERIALS AND METHODS

Drugs and chemicals

Standard drugs were received as drug sample: Phenytoin (Sun Pharmaceuticals India Ltd., Halol, Gujarat, India), Diazepam (Ranbaxy Laboratories Ltd, HMTD textiles, India), Pentylenetetrazole (Sigma-Aldrich, St. Louis, MO63103, USA). All the solvents used for the extraction process were of Laboratory grade and they were purchased from local firms.

Experimental animals

Swiss albino mice of either sex (18 - 25 g) were obtained and acclimatized at the animal house of Advanced Institute of Pharmacy, Palwal, Haryana. Standard conditions were provided to all the animals, that is room temperature 26 ± 1°C, Relative humidity 45 - 55% and

12:12 h light- dark cycle. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Advanced Institute of Pharmacy, palwal (Approval number: IAEC/AIP/2015/ 438 dated 18/12/2015.) and all the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Preparation of Extract

The leaves of *Shorae robusta* were collected from local regions of Dehradoon, Uttranchal. Plant was authenticated at department of botany, Pt. Jawahar Lal Nehru Government College, (Approval No: IAEC/AIP/2015/ 438) Faridabad, Haryana. A sample of which was kept in the department.

Leaves of *S. robusta* dried at room temperature and pulverized to form coarse powder. Extracts were prepared by the method described by Rosenthaler[8]. 500g of powdered leaves was placed in soxhlet apparatus (Perfit, India) and defatted with petroleum ether (60°-80°C) and extracted with methanol and water. This extraction process was continued for about 48 hours or until methanol coming down the siphoning tube became colourless. Aqueous extract was obtained after macerating the powdered leaves (500gm) for 72 hours with occasional stirring. Extracts so obtained were filtered and the filtrate was evaporated using vacuum evaporator (Perfit, India) under reduced pressure at ≤ 50° C. The crude extract obtained after evaporation were stored in desiccator and weighed. Physical nature and percentage yield of extract was recorded. The % yield of extracts was evaluated using the formula:

% Yield= Weight of extract (g)/ Weight of dry powder (g) × 100

The detail of the prepared extracts is mentioned below:

SRME: *S. robusta* leaves methanol extract

SRAE: *S. robusta* leaves aqueous extract

Preliminary Phytochemical Screening

The extracts were dissolved in methanol and were further diluted with distilled water so as to give a concentration of 10 % w/v. The solutions so prepared were subjected to the following tests.

Test for Amino acids & proteins

- Ninhydrin test: 0.1 % solution of Ninhydrin reagent in alcohol was added to 2ml of test solution and observed for the formation of violet-purple color.
- Biuret's test: To 1 ml of test solution, 2 ml of biuret's reagent was added. The mixture was allowed to stand and was observed for development of blue precipitate.
- Millon's reagent test: Millon's reagent was added to the test solution and was observed for development of red color.

Test for Carbohydrates

- Molisch's test: To 2 ml of test solution, 1 ml of Molisch reagent was added. The mixture was warmed and allowed to stand and was observed for formation of violet color ring.
- Benedict's test: To 1 ml of test solution, 2 ml of Benedict's reagent was added. The mixture was warmed, allowed to stand and observed for formation of red precipitate.
- Barford's test: To 1 ml of test solution, 2 ml of Barford's reagent was added. The mixture was boiled for 2 min and allowed to stand and was observed for formation of red cuprous oxide.
- Fehling's test: A few drops of test solution were added to equal quantity of Fehling's solution A & B, the mixture was heated after each addition and was finally observed for the formation of slightly yellow/brown precipitate.

Test for Essential oils

Small quantity of extract was placed on filter paper and heated in an oven for about 30 min. Presence of oil on filter paper was observed.

Test for Alkaloids

- Hagner's reagent (picric acid): To 2 ml of test solution, 0.5 ml of Hagner's reagent was added and observed for the formation of yellow precipitate.
- Wagner's reagent (aqueous iodine solution): To 2 ml of test solution, 0.5 ml of Wagner's reagent was added and observed for the formation of reddish brown precipitate.
- Mayer's reagent (mercury potassium iodide): To 2 ml of test solution, 0.5 ml of Mayer's reagent was added and observed for the formation of cream precipitate.

Test for Flavonoids

- To 2 ml of test solution, magnesium sulphate and few drops of concentrated hydrochloric acid were added and then observed for the formation of orange/red color precipitate.
- Ferric chloride solution: To 2 ml of test solution, 2 ml of ferric chloride was added and observed for the formation of dark blue color.

Test for Glycosides

- Acid hydrolysis was performed on a small portion of plant extract using 6 M HCl for 6 hrs and then extracted with chloroform. Concentrated chloroform extract was subjected to the following tests:

- Libermann-Burchard test: Libermann-Burchard reagent was added to chloroform extract and observed for color development for 15 min.
- Baljet's test: Sodium picrate was added to chloroform extract and observed for color development from yellow to orange to test the presence of cardiac glycosides.
- Legal's test: To chloroform extract, pyridine and sodium nitroprusside was added and then made alkaline and observed for development of green color to test the presence of cardiac glycosides.
- Borntrager's test: To the chloroform extract, ammonia solution was added and observed for development of pink color to test the presence of anthraquinone glycosides.

Test for Saponins

- The test solution was observed for the formation of foam on shaking with water.

Test for Tannins &Phenolic compounds

- Lead acetate solution: 1 ml of lead acetate solutions (10 %) was added to 2 ml of test solutions and then observed for the formation of white precipitate.
- Gelatin solution test: 1 % of gelatin solution containing 10 % sodium chloride was added to 2 ml of test solution and then observed for the formation of white precipitate.

Chemical test for Triterpenoids

- Salkwaski Test: to 2 ml of extract 5 drops of conc sulphuric acid added, sheken well nad allowed to stand. Appearance of greenish blue colour indicates the presence of triterpenoids
- Libermann Burchard Tast: To 2ml oftest solution 10 drops of acetic anhydride was added and mixed well. To this 5ml of conc sulphuric acid was added from the sides of test tube, appearance of greenish blue colour indicates the presence of triterpenoids.

Test for Ascorbic acid

- To 2 % w/v of test solution, 1 drop of freshly prepared 5 % w/v solution of sodium nitroprusside and 2 ml of dilute sodium hydroxide solution were added. 0.6 ml of hydrochloric acid was added drop wise and stirred; yellow color turns blue.
- To 2 % w/v solution of test, 0.1 g of sodium bicarbonate and about 20 mg of ferrous sulphate added, shake and allowed for standing; a deep violet color was produced. Then 5 ml of 1M sulphuric acid was added; the color disappeared.

Preliminary phytochemical Screening was done according to standard procedures[9].

Acute toxicity studies

Oral toxicity of alcoholic extracts of *S. robusta* was estimated by using mice of weight 18 - 25 g. Prior to experiment, animals were kept fasting for 5 hrs. Animals were administered with single dose of extract and observed for their mortality during 48 h study period (short term) toxicity[10]. Gradually increased dose of s. robusta extract was administered to mice. LD_{50} was calculated as per OECD guidelines 425[11].

Pentylenetetrazole (PTZ) induced seizure test

Swiss albino mice were divided into 5 groups with 6 animals each, either group served as control (NS 0.1%), standard (diazepam 4 mg/kg) and plant extracts (*S. robusta* 100, 300 and 500 mg/kg) all groups receive PTZ (100 mg/kg) after 30 min of standard/extracts respectively except control group which receive NS before the administration of PTZ. All the groups receive PTZ through intraperitonial route of administration. Each animal was individually investigated for following perameters i.e. onset of seizure, duration of seizure and Recovery or Death. These parameters were initially recorded for 30 min and at end of 24 hrs[12]

Maximal Electroshock induced convulsion

Maximal Electroshock induced convulsion was done by using swiss albino mice with body weight of 18 to 25 gm. Animal were divided into 5 groups with 6 rats in each, as discussed in PTZ method. Control group received normal saline, standard group received phenytoin (25mg/kg) orally and test group received single dose of 100, 300 and 500mg/kg respectively. 30 min after the administration of extract in control group, phenytoin in standard group in different dose of extracts in different test group respectively, experiment was started. Maximal electroshock (MES, inco) of 50 mA current for 0-2sec was given to each animal individually through ear electrode to induce convulsions to drug treated and control group [13].

Strychnine Induced Convulsions

Mice were randomly selected and named as control, test and standard group with same criteria as that of PTZ model. The control groups receive strychnine (2mg/kg) after 30 min of administration of normal saline. Standard group was administered with diazepam (5mg/kg) and then with strychnine (2mg/kg) after 30 min. test group received strychnine (2mg/kg) 30 min after the administration of 100, 300 and 500mg/kg of S. robusta extracts respectively. Any mouse that did not convulse within 30 min after strychnine administration was considered protected [14].

Picrotoxin induced convulsions

Mice were randomly allotted as described in strychnine induced method. Picrotoxine (3.5mg/kg) was used to induce seizure using same protocol as discussed in strychnine induced method [15].

RESULTS AND DISCUSSION

% age Yield of Extracts

$$\%\text{age of SRME(shorea robusta methanolic extract)} = \frac{\text{Weight of Extract}}{\text{Weight of Powder}} \times 100$$

i. e.

$$= \frac{54}{500} \times 100$$

= 10. 80

$$\%\text{age yield of SRAE (shorea robusta aqueous extract)} = \frac{\text{Weight of Extract}}{\text{Weight of Powder}} \times 100$$

i.e.

$$= \frac{41.5}{500} \times 100$$

= 8.30

Preliminary Phytochemical Screening

Preliminary phytochemical screening of SRME shows the presence of Flavonoids, Alkaloids, Saponins, Tannins Anthraquinones, Sterols, Glycosides, Resins, Tryterpins and Reducing Sugars. Presence of flavonoids in the extract may be responsible for anticonvulsant effect.[16] Phytochemical screening of *S. robusta* shows the presence of following constituents in methanolic as well as aqueous extract. (Table-1)

Table 1: Different phytoconstituents of methanolic and aqueous extraction of *S. robusta*

S. No	Tests	Methanol extract	Aqueous Extract
1.	Alkaloid	Absent	Present
2.	Glycoside	Present	Absent
3.	Saponin glycoside	Present	Absent
4.	Steroidal triterpenoid (Liberman and salkowoski)	Present	Present
5.	Flavonoids	Present	Absent
6.	Amino acid	Absent	Present
7.	Carbohydrates	Present	Absent
8.	Tannin & Phenolic compounds	Present	Absent
9.	Fixed oils	Absent	Absent

Acute Toxicity Study

The 50% of total mice were dead at the dose of 19.7 g/kg (LD_{50} = 19.7 gm/kg). The dose of 47.5 g/kg cause death of 100% animal.

Experimental Models

Epilepsy may be defined as a group of chronic central nervous system disorder characterized by seizures and may be associated with unconsciousness and body movements. MES and PTZ is most commonly and widely accepted animal model for the evaluation of anticonvulsant activity. Strychnine and Picrotoxin are not used frequently as death rate is high.

PTZ induced convulsion

In case of chemical models, PTZ induced seizures are most commonly used animal model. PTZ induced convulsions are similar to petitmal seizures and generalized seizure. Most of agents effective against petitmal seizure act by reducing t-type calcium channel current and by enhancing γ-amino butyric acid (GABA) and benzodiazepine receptor which are mediated by benzodiazepine and phenobarbiton neurotransmitter. Drugs that block glutaminergic excitation mediated by NMDA receptor viz. Felbamet, have anticonvulsant activity against PTZ induced convulsions. In present study SRME at the dose 500mg/kg shows significant activity against PTZ induced convulsions. Whereas SRAE reduced the onset of convulsion but ineffective in decreasing duration of action. In this model, 300 and 500mg/kg of *S. robusta* methanolic extract delayed the onset of clonic seizure especially at the dose of 500mg/kg, significantly ($P < 0.01$). Similarly *S.robusta* extract delayed the onset of tonic seizure especially at the dose of 500mg/kg. Standard drug diazepam inhibits onset of tonic seizure and death caused by convulsion. Death rate and tonic seizure were reduced by *S. robusta* at dose of 500mg/kg. (Table.2). Anticonvulsant activity of SRAE on PTZ-induced seizures: the analysis of table-3 suggests that there is no marked significance of results of found in aqueous extraction of S. robusta.

Maximal Electroshock induced convulsion

In present study the result of MES animal model suggests significant anticonvulsant activity of SRME at the dose 500mg/kg. SRME at the dose 500mg/kg show decrease in duration of flexon, extensor and stupor phase of tonic clonic seizures. These are also decreased by 100 and 300 mg/kg dose as well but at lesser extent. But SRAE was little insignificant in controlling seizures in this model. From the analysis of result (Table.4) it was observed that extract of *S. robusta* with the dose 100, 300 and 500mg/kg of body weight reduced all the phases of convulsion.

Table 2: Anticonvulsant activity of SRME on PTZ-induced seizures

Group	Treatment	Num of Animals	Onset of convulsion (sec)	Duration of Convulsion (sec)	DEATH % age
Group-I	Control (Saline 0.9%, per oral)	6	58±0.10	287±0.03	5/6 (83.33)
Group-II	Standard (diazepam 4 mg/kg intraperitonealy)	6	236 ± 0.12**	27 ± 0.10**	0/6 (0.00)
Group-III	SRME 100 mg/kg per oral	6	42 ± 0.48	237 ± 0.67	3/6 (50.00)
Group-IV	SRME 300 mg/kg per oral	6	87 ± 0.50*	196 ± 0.54*	2/6 (33.33)
Group-V	SRME 500 mg/kg per oral	6	100 ± 0.76**	127 ± 0.65**	1/6 (16.66)

(All values expressed as mean±SEM; n=6 mice in each group, by one-way ANOVA followed by Dunneet's t- Test (compared with control group) *p<0.05, **p<0.01 and ***p<0.001.)

Table 3: Anticonvulsant activity of SRAE on PTZ-induced seizures

Group	Treatment	Num of Animals	Onset of convulsion (sec)	Duration of Convulsion (sec)	DEATH % age
Group-I	Control (Saline 0.9%, per oral)	6	58 ± 0.10	287 ± 0.03	5/6 (83.33)
Group-II	Standard (diazepam 4 mg/kg intraperitonealy)	6	338 ± 0.14**	27 ± 0.11**	0/6 (0.00)
Group-III	SRME 100 mg/kg per oral	6	40 ± 0.48	280 ± 0.67	3/6 (50.00)
Group IV	SRME 300 mg/kg per oral	6	64 ± 0.50	278 ± 0.54	2/6 (33.33)
Group V	SRME 500 mg/kg per oral	6	76 ± 0.76	269 ± 0.65	1/6 (16.66)

(All values expressed as mean ± SEM; n=6 mice in each group, by one-way ANOVA followed by Dunneet's t- Test (compared with control group) *p<0.05, **p<0.01 and ***p<0.001.)

Table 4: Effect of SRME of *S. robusta* in Maximal Electroshock induced convulsion

Group	Treatment	Num of Animals (n)	Duration of Convulsion (Time in Sec)			DEATH % age
			Flexion	Extensor	Stupor	
Group-I	Control (0.9% Normal saline p.o)	6	27 ± 0.24	52 ± 0.07	200 ± 0.50	4/6 (66.66)
Group-II	Standard (Phenytoin 25g/kg i.p)	6	11 ± 0.36**	_	_	0/6 (0.00)
Group-III	SRME (100 mg/kg p.o)	6	20 ± 0.76	27 ± 0.76	130 ± 0.32	2/6 (33.33)
Group-IV	SRME (300 mg/kg p.o)	6	15 ± 0.38	25 ± 0.76	116 ± 0.50	1/6 (16.66)
Group-V	SRME (500 mg/kg p.o)	6	14 ± 0.38*	19 ± 0.07*	112 ± 0.30*	1/6 (16.66)

(All values expressed as mean± SEM; n=6 mice in each group, by one-way ANOVA followed by Dunneet's t- Test (compared with control group) *p<0.05, **p<0.01 and ***p<0.001.)

Table 5: Anticonvulsant activity of SRAE on MES-induced seizures

Group	Treatment	Num of Animals (n)	Duration of Convulsion (Time in Sec)			DEATH % age
			Flexion	Extensor	Stupor	
Group-I	Control (0.9% Normal saline p.o)	6	27 ± 0.24	52 ± 0.07	200 ± 0.31	4/6 (66.66)
Group-II	Standard (Phenytoin 25mg/kg i.p)	6	14 ± 0.36**	_	_	0/6 (0.00)
Group-III	SRAE (100 mg/kg p.o)	6	21 ± 0.76	19 ± 0.76	135 ± 0.32	2/6 (33.33)
Group-IV	SRAE (300 mg/kg p.o)	6	20 ± 0.38	20 ± 0.76	127 ± 0.50	1/6 (16.66)
Group-V	SRAE (500 mg/kg p.o)	6	19 ± 0.38	19 ± 0.07	118 ± 0.30	1/6 (16.66)

(All values expressed as mean ± SEM; n=6 mice in each group, by one-way ANOVA followed by Dunneet's t- Test (compared with control group) *p<0.05, **p<0.01 and ***p<0.001.)

Strychnine Induced Seizures

Strychnine has been reported to produce its convulsant effect by antagonizing and inhibitory reflexes of glycine in spinal cord and brain stem and thus increase the spinal reflex[17]. The results of srychnine model shows little increase in onset of tonic and clonic seizure in both SRME and SRAE treated groups. Doses of extract of *S.robusta* i.e 100, 300and 500mg/kg were not able to inhibit seizures evoked by strychnine (4mg/kg) as done by standard drug diazepam (10mg/kg) but delay in onset of tonic clonic seizures was shown by 500mg/kg of extract. (Table-6 & 7).

Table 6: Effect of SRME of *S. robusta* in Strychnine induced seizures

S. No.	Group/Traetment	Onset of clonic seizures in min ± S. E.M.	Onset of tonic seizures in min ± S. E.M.	DEATH % age
1	Control group (Normal Saline)	2.2 ± 0.2	2.3 ± 0.2	6/6 (100%)
2	Standard group (Phenytoin 25mg/kg)	6.9 ± 0.5**	8.6 ± 0.8**	3/6 (50%)
3	Test Group-I (*S. robusta* 100mg/kg)	2.4 ± 0.3	3.5 ± 0.4	6/6 (100%)
4	Test Group-II (*S. robusta* 300mg/kg)	2.7 ± 0.3	3.6 ± 0.4	5/6 (83.33%)
5	Test Group-III (*S. robusta* 500mg/kg)	3.3 ± 0.4*	6.01 ± 0.7**	4/6 (66.66%)

(All values expressed as mean ± SEM; n=6 mice in each group, by one-way ANOVA followed by Dunneet's t- Test (compared with control group) *p<0.05, **p<0.01 and ***p<0.001.)

Table 7: Effect of SRAE of *S. robusta* in Strychnine induced seizures

S. No.	Group/Traetment	Onset of clonic seizures in min ± S. E.M.	Onset of tonic seizures in min ± S. E.M.	DEATH % age
1	Control group (Normal Saline)	4.2 ± 0.4	4.3 ± 0.4	6/6 (100%)
2	Standard group (Phenytoin 25mg/kg)	8.9 ± 0.7**	10.6 ± 0.9**	2/6 (33.33%)
3	Test Group-I (*S. robusta* 100mg/kg)	4.7 ± 0.5	5.5 ± 0.6	6/6 (100%)
4	Test Group-II (*S. robusta* 300mg/kg)	5.5 ± 0.4	5.6 ± 0.7	5/6 (83.33%)
5	Test Group-III (*S. robusta* 500mg/kg)	6.3 ± 0.4*	8.01 ± 0.9**	4/6 (66.66%)

(All values expressed as mean ± SEM; n=6 mice in each group, by one-way ANOVA followed by Dunneet's t- Test (compared with control group) *p<0.05, **p<0.01 and ***p<0.001.)

Picrotoxin induced seizures

Picrotoxin is a known competitive GABA antagonist at receptor GABAa [18]. As GABA is major inhibitory neurotransmitter and its inhibition cause over excitation of nerves of brain and spinal cord which is major factor underlying that evoke epilepsy. Similar results were observed in both SRME and SRAE treated groups i.e delayed onset of tonic and clonic seizure. As compared to control group all doses of extract i.e 100, 300 and 500 mg/kg shows delayed onset of tonic and clonic seizure against picrotoxin (3.5mg/kg), but as compared to standard group delaying in onset of tonic and clonic seizure were less significant. Though the 500 mg/kg dose of extract of S. robusta delayed the onset of tonic and clonic seizure up to little extant (Table 8 & 9). Mostly, drugs that increase GABA currents are known to show evidence of anticonvulsant activity against PTZ, but may be weak or ineffective against picrotoxin-induced convulsions [19].

Table 8: Effect of alcoholic extract of *S. robusta* in Picrotoxin induced seizures

S. No.	Group/Treatment	Onset of clonic seizures in min ± S. E.M.	Onset of tonic seizures in min ± S. E.M.	DEATH % age
1	Control group (Normal Saline)	5.9 ± 0.2	6.5 ± 0.3	6/6 (100%)
2	Standard group (Phenytoin 25mg/kg)	17.6 ± 1.7**	20.7 ± 2.8**	2/6 (33.33%)
3	Test Group-I (*S. robusta* 100mg/kg)	6.6 ± 1.4	10.3 ± 1.7	6/6 (100%)
4	Test Group-II (*S. robusta* 300mg/kg)	6.9 ± 1.4	10.6 ± 1.7	4/6 (66.66%)
5	Test Group-III (*S. robusta* 500mg/kg)	7.4 ± 1.2	12.3 ± 1.7*	4/6 (66.66%)

(All values expressed as mean±SEM; n=6 mice in each group, by one-way ANOVA followed by Dunneet's t- Test (compared with control group) *p<0.05, **p<0.01 and ***p<0.001)

Table 9: Effect of SRAE of *S. robusta* in Picrotoxin induced seizures

S. No.	Group/Traetment	Onset of clonic seizures in min ± S. E.M.	Onset of tonic seizures in min ± S. E.M.	DEATH % age
1	Control group (Normal Saline)	9.9 ± 0.4	10.5 ±0.7	5/6 (83.33%)
2	Standard group (Phenytoin 25mg/kg)	21.6 ± 1.9**	24.7 ± 3.2**	1/6 (16.66%)
3	Test Group-I (*S. robusta* 100mg/kg)	13.6 ± 1.7	14.5 ± 2.1	6/6 (100%)
4	Test Group-II (*S. robusta* 300mg/kg)	13.9 ± 1.8	14.9 ± 2.1	5/6 (83.33%)
5	Test Group-III (*S. robusta* 500mg/kg)	15.4 ± 1.6	18.6 ± 2.1*	5/6 (83.33%)

(All values expressed as mean± SEM; n=6 mice in each group, by one-way ANOVA followed by Dunneet's t- Test (compared with control group) *p<0.05, **p<0.01 and ***p<0.001.)

CONCLUSION

From this study it is clearly understood that SRME in highly effective as compared to SRAE in all the Animal models and suggests that anticonvulsant action of SRME.

On the other hand SRAE was little significant in PTZ and MES models but slightly less effective in Picrotoxin and strychnine induced convulsion models. It suggests a different mechanism attributed to chemical constituents present in the extracts.

The obvious insensitivity of picrotoxin to SRME may be related to the target of its action at the chloride ion gate, which is not easily accessible to most anticonvulsant drugs [20]. Thus, the failure to gain access to the closed chloride ion gate in order to reopen it might explain the lower activity of SRME to prevent seizures due to picrotoxin [21]. Similar results were obtained by *Annafi et al*.*

Therefore it can be concluded that anticonvulsant activity of SRME is due to the blocking of seizure spread by Na+ glutaminergic excitation and NMDA receptor mediation. This study also suggests that SRME is also effective against tonic clonic seizures. Thus the plant *S. robusta* posses anticonvulsant activity aganst MES, PTZ, Strychnin and picrotoxine animal models.

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Source of Support: Nil, **Conflict of Interest:** None.