Original Article

Exploring the protective effects of aqueous extracts of *Ruta Chalepensis* Linn. On drug induced seizures in animal models

Ramdas Bhat[1], Hemalatha C H[2], A R Shabaraya[3]

From, [1]Assistant Professor, [2]PG Scholar, Department of Pharmacology, [3]Principle and Head of the Department of Pharmaceutics, Srinivas College of Pharmacy, Valachil, Post Farangipete, Mangalore, Karnataka, India - 574143.

ABSTRACT

Background and Objective: Convulsion is a sudden, involuntary muscle contraction causing rapid and repetitive movements of the body. *Ruta Chalepensis* Linn. In is a medicinal plant used to treat a wide range of disorders including seizure. But, the anticonvulsant activity of this plant has not been studied in deep. We therefore sought to evaluate the anticonvulsant activity of an aqueous leaf extract of *Ruta Chalepensis* Linn. On convulsing induced in mice. **Method:** The anticonvulsant activity of an aqueous leaf extract of *Ruta Chalepensis* Linn. On convulsing induced in mice, by strychnine nitrate (2.0 mg/kg) and Pentylenetetrazole (PTZ) (80 mg/kg) in mice. Chronic study was conducted using low, medium and high doses of ARC (250, 500 and 750mg/kg respectively). The above-mentioned doses were administered daily once for a period of 7 consecutive days. Aqueous extract (250, 500 and 750 mg/kg), diazepam (10mg/kg intraperitoneally or oral.), and distilled water (10 ml/kg, i.p) were administered before induction of seizures. **Results:** In strychnine induced convulsion test the onset of seizure was significantly delayed in the animals treated with *Ruta Chalepensis* Linn leaf aqueous extract at 750mg/kg. In PTZ induced convulsion model the onset of seizure was significantly delayed in the animals treated with *Ruta Chalepensis* Linn leaf aqueous extract at a dose of 750mg/kg. **Conclusion:** As a result of foregoing *Ruta Chalepensis* Linn leaf aqueous extract has considerable diuretic activity at higher doses.

Key words: Aqueous extracts of *Ruta chalepensis* Linn (AERC), Strychnine-induced convulsion test, Pentylenetetrazole (PTZ) induced Seizures, Epilepsy, Glycine, GABAnergic.

Epilepsy is a neurological disorder characterized by recurrent and unpredictable seizures caused by sudden bursts of abnormal electrical activity in the brain. Seizures can be of different types, including tonic-clonic, myoclonic, absence, and others, and are often treated with anticonvulsant medications to reduce the risk of seizures [1]. Despite the availability of different medications, there are still a considerable number of patients who experience inadequate seizure control or suffer from medication side effects. This has led to a search for alternative treatment options, including herbal remedies that have been used for centuries to treat seizures [2]. *Ruta chalepensis* Linn. Is a medicinal plant that has been traditionally used for various ailments, including seizures.

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The plant contains several bioactive compounds, such as alkaloids, flavonoids, and coumarins, which have various pharmacological activities, including anticonvulsant, anti-inflammatory, and analgesic effects [3]. Pentylenetetrazole (PTZ), strychnine are commonly used compounds to induce convulsions in animal models [4]. Strychnine is a potent convulsant that acts by blocking the inhibitory neurotransmitter glycine in the spinal cord, leading to increased excitability and ultimately, convulsions [5]. While PTZ-induced convulsions are mainly mediated by the inhibition of the neurotransmitter GABA, strychnine -induced convulsions are not mediated by GABA [6], making them a useful model for investigating alternative pathways and mechanisms involved in seizures. The use of animal models in the study of convulsions and seizures has been widely accepted and employed in the field of epilepsy research [7].

Correspondence to: Ramdas Bhat, Assistant Professor, Department of Pharmacology, Srinivas College of Pharmacy, Valachil, Post Farangipete, Mangalore, Karnataka, India-574143. **Email:** ramdas21@gmail.com

Animal models allow for the investigation of various factors that contribute to the development of convulsions, such as age, sex, species, diet, water, day/light cycle, temperature, preparation dose, and route of administration. Furthermore, animal models allow for the evaluation of the efficacy and safety of potential anticonvulsant treatments [8, 9]. The aqueous extracts of *Ruta chalepensis* Linn. Have been reported to have several pharmacological activities, including anticonvulsant effects, which makes them a promising candidate for further investigation. The evaluation of the potential protective effects of *Ruta chalepensis* Linn. Against both PTZ and strychnine induced convulsions in animal models may provide new insights into the mechanisms of action involved in these seizures and identify new therapeutic strategies for the treatment of epilepsy.

METHODOLOGY

Animals: The following study used mice of both sexes weighing around 18-22g. The animals were fed a standard pelleted diet (Lipton India Ltd., Mumbai) and distilled water ad libitum under a constant 12 hours light and dark cycle. Prior to the experiment, all animals were housed in laboratory conditions for 5 days. The animals are now divided into 5 groups containing 6 animals each. All experiments were carried out in accordance with the ethical standards.

Plant extract: Plant was collected in Mangalore and authentication was done by Botanist, Pilikula Nisargadhama, Vamanjoor. The leaves are separated shade dried for one day and the aqueous drug extract was prepared by steam distillation. The extract was suspended in 1% tween 80, and was administered orally to the animals by gastric intubation using force feeding needle. Dose was selected as 250mg/kg and 500mg/kg and 750mg/kg as lower, intermediate and higher dose respectively based on the acute toxicity study (LD50).

Study type: Screening of *Ruta chalepensis* Linn leaf aqueous extract for its anticonvulsant activity using experimental animals.

Screening models used:

a) Strychnine-induced Convulsions Test
b) Pentylenetetrazole (PTZ) -induced Seizures.

Study site: Department of Pharmacology, Srinivas College of pharmacy, Valachil, Mangalore.
Sample size: 60 mice were selected for the entire study
Study Duration: 7 days
Inclusion criteria: Mice of both sex having weight of 18-22g was included in study
Exclusion criteria: Mice above 25g was excluded from the study.

Experimental Design

Acute toxicity studies: Acute toxicity study was performed in accordance with OECD guidelines. No adverse effect or mortality was detected in mice up to 2 gm/kg, p.o of *Ruta chalepensis* Linn during the 24 to 72 hrs observation periods. The rats were continually monitored for 5 hours during this time for any obvious behavioral, neurological, or autonomic toxic effects, mortality after 24 to 72 hrs (Table 1 and 2).

Strychnine induced convulsion: The mice utilized for this study were groups of six of either sex with a weight of between 18-22 respectively. They were now treated with Diazepam 10 mg/kg which is a standard drug. One hour after the administration of the standard drug the mice were injected with 2 mg/kg Strychnine nitrate i.p the time until occurrence of tonic extensor convulsions and death noted during a 1 h period. With this dose of strychnine convulsions were observed in 80% of the controls. The animals employed are mice of either sex that weigh between 18 and 22 g. The animals are divided into 6 groups and 6 groups receive either the test substance or the reference medication through injection, intravenously, or orally. As a control, six mice from another group are used. Thirty minutes following an intravenous injection, sixty minutes following a subcutaneous injection PTZ is subcutaneously given at an 80 mg/kg dose. Each animal is housed in its own plastic cage for a one-hour observation period. There are records of seizures and tonic-clonic convulsions. Convulsions must be present in at least 80% of the animals in the control group.

Table 1: Grouping of animals in strychnine induced convulsion

Group	Treatment	Dose	No. of animals	Parameters to be assessed
Group I (control)	Saline + Strychnine	10ml/kg + 2mg/kg	6	
Group II (standard)	Diazepam + Strychnine	10mg/kg + 2mg/kg	6	Percentage inhibition of
Group III (Low Dose)	AERC + Strychnine	250mg/kg + 2mg/kg	6	seizures relative to
Group IV (Moderate Dose)	AERC + Strychnine	500mg/kg + 2mg/kg	6	control
Group V (High Dose)	AERC + Strychnine	750mg/kg + 2mg/kg	6	

Table 2 - Pentylenetetrazole-induced Seizures (PTZ)

Group	Treatment	Dose	No. of animals	Parameters to be assessed
Group I (control)	Saline + Pentylenetetrazole	10ml/kg + 80mg/kg	6	
Group II (standard)	Diazepam + Pentylenetetrazole	10mg/kg + 80mg/kg	6	Percentage inhibition of seizures relative to control
Group III (Low Dose)	AERC + Pentylenetetrazole	250mg/kg + 80mg/kg	6	
Group IV (ModerateDose)	AERC + Pentylenetetrazole	500mg/kg + 80mg/kg	6	
Group V (High Dose)	AERC + Pentylenetetrazole	750mg/kg + 80mg/kg	6	

Statistical analysis: All the data were expressed in mean ± SEM. The significance of differences in mean between control and treated animals for different parameters determined by one way ANOVA followed by Dunnett's multiple comparison test. Significance for difference between groups were evaluated for student's t-test to come to final conclusion.

RESULT AND DISCUSSION

AERC leaves were screened for anticonvulsant activity using Strychnine and PTZ induced convulsion model in mice. Chronic study was conducted using low, medium and high doses of ARC (250,500& 750mg/kg respectively). The above-mentioned doses were administered daily once for a period of 7 consecutive days.

It was observed that lower dose (250mg/kg) of AERC did not produce significant anticonvulsant effect as compared to control. But medium and high doses (500&750mg/ kg respectively) exhibited a significant anticonvulsant effect by increasing onset time of seizures and reducing the duration of tonic-clonic seizures (Figure 1 – 3).

Strychnine induced convulsion: The mice were given *Ruta chalepensis* leaf extract in three different doses considerably delayed the onset of seizures. Of the 6 animals tested, 4 survived at the higher dose (750 mg/kg), 2 survived at the intermediate level (500 mg/kg), and none survived at the lower dose. For higher and moderate doses, the percentage protection was 66.66 and 33.33 respectively (Table 3).

Table 3: Effect of AERC on Strychnine induced convulsion.

Treatment	Onset of tonic clonic convulsion Mean ± SEM	Status of animal (1hr) (No. of animals Alive)	% Protection
Control	254.50 ± 0.29	0	0
Diazepam	481.72 ± 0.87***	All	100
AERC (250mg/kg)	252.83 ± 0.57	0	0
AERC (500mg/kg)	276.55 ± 0.89	2	33.33
AERC (750mg/kg)	276.50 ± 1.52**	4	66.66

All values are expressed as (Mean ± S.E.M), n=6, **p≤0.01, ***p≤0.001 when compared with control. (Statistically analyzed by ANOVA allowed by Dunnett's-test).

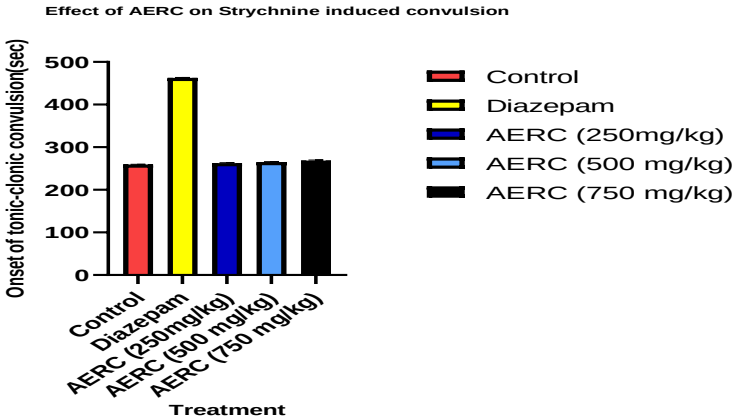

Fig. 1: Effect of AERC on Strychnine induced convulsion.

AERC leaves were tested for their ability to prevent convulsions induced by strychnine in mice. A chronic study was conducted using three different doses of AERC (250mg/kg, 500mg/kg, and 750mg/kg), administered once daily for seven consecutive days. The lower dose did not show a significant anticonvulsant effect compared to the control group, but the medium and high doses (600mg/kg and 800mg/kg) demonstrated a significant effect by

delaying the onset of seizures and reducing the duration of tonic-clonic seizures. The protective effect of the medium and high doses of ARC was 33.33% and 66.66% for up to one hour after administration, respectively, while the lower dose showed no protective effect. The standard drug diazepam (5mg/kg) exhibited a significant anticonvulsant activity and provided 100% protection (Table 4).

PTZ induced convulsion: *Ruta Chalepensis* leaf extract, 750 mg/kg, considerably delayed the onset of seizures in mice treated in the PTZ-induced convulsion model, and it significantly decreased the length of tonic-clonic seizures for both the moderate and higher dosages of test as well as standard. In the test with the higher dose, only two of the six animals showed signs of survival, whereas one animal did so for the test with the moderate level and none at the low amount. The percentage of protection was 33.33 for higher doses and 16.66 for moderate doses, respectively.

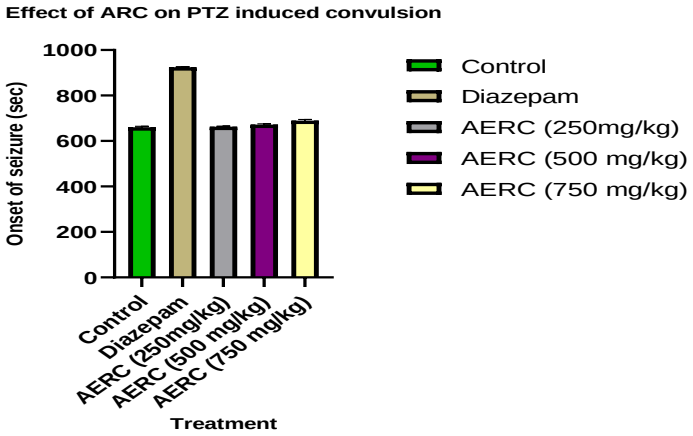

Fig. 2: Effect of AERC on PTZ induced convulsion.

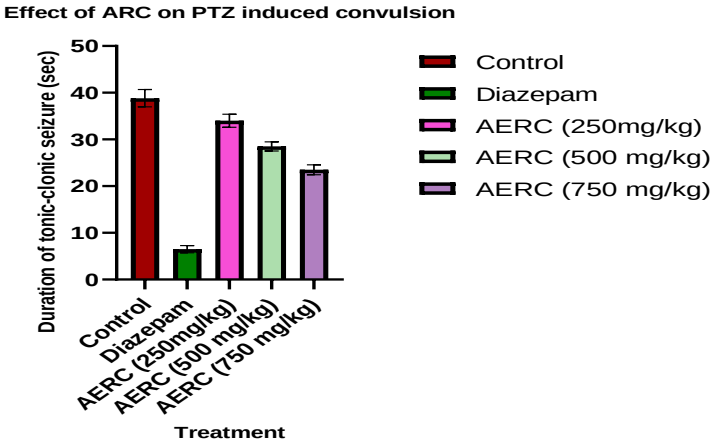

Fig. 3: Effect of AERC on PTZ induced convulsion

Table 4: Effect of AERC on PTZ induced convulsion.

Treatment	Onset of Seizure (Sec) Mean± SEM	Duration of Tonic Clonic Seizure (Sec) Mean± SEM	Status of Animal (No. of Animals Alive)	% Protection(1hr)
Control	660.17 ± 4.01	38.83 ± 1.85	0	0
Diazepam	924.67 ± 2.65***	6.50 ± 0.76***	All	100
AERC (250mg/kg)	663.33± 2.30	34.00 ± 1.39	0	0
AERC (500mg/kg)	671.67 ± 4.02	28.5 ± 0.99***	1	16.66
AERC (750mg/kg)	689.83 ± 4.40***	23.50 ± 1.05***	2	33.33

All values are expressed as (Mean ± S.E.M), n=6, ***p≤0.001 when compared with control. (Statistically analyzed by ANOVA allowed by Dunnett's-test).

AERC leaves were tested for anticonvulsant activity in mice using the PTZ induced convulsion model. Three different doses of ARC (250mg/kg, 500mg/kg, and 750mg/kg) were administered once daily for seven

consecutive days. The lower dose did not show significant anticonvulsant activity compared to the control group, but the medium and high doses showed a significant effect by delaying the onset of seizures and reducing the duration of tonic-clonic seizures. The protective effect of the medium and high doses of AERC was 83.33% and the lower dose was 33.33% for up to one hour after administration. The standard drug, diazepam (1mg/kg), exhibited significant anticonvulsant activity and provided 100% protection.

Ruta chalepensis is an ancient medicinal plant still used in the traditional medicine of many countries as a laxative, anti-inflammatory, analgesic, anti-spasmodic, abortifacient, anti- epileptic, emmenagogue and for dermatopathy treatment. The main aim of this work is to investigate the antiepileptic activity of Ruta chalepensis aqueous leaf extracts in experimental animals [10]. The antiepileptic activity of the Ruta chalepensis leaf extracts has not yet been studied for anti-convulsant activity. He exact mechanism underlying antiepileptic activity of *Ruta chalepensis* Linn. Leaf extract is not clear but it may be apparently related to active compounds present in *Ruta chalepensis* are reviewed. Chemical studies have reported the presence of several flavonoids, alkaloids, phenols, amino acids, furanocoumarins and saponins in the leaves of *Ruta chalepensis* Linn [11]. The results indicate that aqueous leaf extract of *Ruta chalepensis* Linn. May have an antiepileptic like effect. However further experiments are necessary to confirm this hypothesis.

Future Prospectives

Research in the field of plant-based anticonvulsants has yielded promising results in recent years. However, it is important to note that this research is still in its early stages, and more studies are needed to determine the safety and efficacy of these treatments. Nonetheless, the discovery of new plant-based anticonvulsants has the potential to lead to the development of new and more effective therapies for convulsions. Further research and clinical trials will be necessary to fully evaluate the therapeutic potential of these plant-based treatments.

CONCLUSION

In six meticulously planned studies involving laboratory animals, the aqueous leaf extract of *Ruta chalepensis* Linn. Showed strong anticonvulsant action, indicating the possibility of using it to treat different kinds of epilepsy. By using actual measurement and statistical validation, the results were validated and observer bias was removed. Also, the chemical components of the extract were determined. These findings give a scientific basis for the extract's therapeutic efficacy even if more research on human patients is required.

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REFERENCES

1. Stafstrom CE, Carmant L. Seizures and epilepsy: an overview for neuroscientists. Cold Spring Harbor perspectives in medicine. 2015 Jun 1; 5(6): a022426.
2. Ventola CL. Epilepsy management: newer agents, unmet needs, and future treatment strategies. Pharmacy and Therapeutics. 2014 Nov; 39(11): 776-77.
3. Al-Ghamdi AY, Fadlelmula AA, Abdalla MO. Total phenolic content, antioxidant and antimicrobial activity of *Ruta chalepensis* L. leaf Extract in Al-Baha area, Saudi Arabia. Jordan J Biol Sci. 2020; 13: 675-80.
4. Annafi OS, Umukoro S, Eduviere AT. Evaluation of the anticonvulsant and anxiolytic potentials of methyl jasmonate in mice. Scientia Pharmaceutica. 2014 Sep; 82(3): 643-54.
5. Salim M. A, Ammoo AM, Ali MH, et al. Antiepileptic Effect of Neuroaid® on Strychnine-Induced Convulsions in Mice. Pharmaceuticals. 2022 Dec; 15(12): 1468-69.
6. Shimada T, Yamagata K. Pentylenetetrazole-induced kindling mouse model. JoVE (J of Visualized Experiments). 2018 Jun 12(136): e56573.
7. Kandratavicius L, Balista PA, Lopes-Aguiar C, et al. Animal models of epilepsy: use and limitations. Neuropsychiatric dis. and treat. 2014 Sep 9: 1693-705.
8. Wang Y, Wei P, Yan F, et al. Animal models of epilepsy: a phenotype-oriented review. Aging and Disease. 2022 Feb; 13(1): 215-16.
9. Reddy DS, Kuruba R. Experimental models of status epilepticus and neuronal injury for evaluation of therapeutic interventions. International j of molecular sciences. 2013 Sep 5; 14(9): 18284-318.
10. Althaher AR, Oran SA, Bustanji YK. Phytochemical analysis, in vitro assessment of antioxidant properties and cytotoxic potential of Ruta chalepensis L. essential oil. J of Essential Oil Bearing Plants. 2020 Nov 1; 23(6): 1409-21.
11. Szewczyk A, Grabowski M, Zych D. *Ruta chalepensis* L. In Vitro Cultures as a Source of Bioactive Furanocoumarins and Furoquinoline Alkaloids. Life 2023 Feb 6; 13(2): 457-8.

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