Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [6] May 2023 : 61-66

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of Anticonvulsant property of *Flacourtia indica in* MES and STN Induced Seizures in Rats

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ABSTRACT
To evaluate the anticonvulsant and antioxidant properties of ethanolic extract of Flacourtia indica using two animal models maximal electroshock (MES), and strychnine nitrate (STN) for inducing seizures in rats. In the entire two animal models MES, STN, each model were included 4 groups, in which albino rats (n=6) were used in each group. First group was considered as control, 2nd group as standard where Phenytoin 25mg/kg, diazepam 4 mg/kg is administered, 3rd and 4th served as test groups which were treated with ethanolic extract of Flacourtia indica (EETM) 200 and 400 mg/kg respectively. In all the three experimental animal models, all the groups were treated for 14 days. On the last day i.e., 14th day after completion of all drugs administration in all two animal models which is total 8 groups of rats, within 30-40 minutes seizures were induced by exposing them to a shock of 150 mA with convulsiometer using ear electrodes for 2 seconds in MES model, and 2 mg/kg of Strychnine (STN) model. Anticonvulsant activity was appreciated better only after abolition of hind limb tonic extension (HLTE) in Maximal electroshock (MES) model and by measuring the duration of seizures and latency induced seizure threshold in the STN experimental rat models. In MES model, EEFI at a dose of 400 mg/kg abolishes complete HLTE in the rats, similarly at the same dose observed prolonged latency in the onset of seizures in STN experimental animal models. It is concluded that EEFI has shown effective anticonvulsant activity in these animal models as it abolishes HLTE in MES model and delayed the latency of seizure threshold in STN models.
Keywords: *Flacourtia indica, Anticonvulsant activity, Antioxidant, Maximal electroshock (MES), Strychnine (STN), and Diazepam.*

Received 09.03.2023 Revised 14.04.2023 Accepted 21.05.2023

INTRODUCTION

Epilepsy a group of disorders characterized by recurrent spontaneous seizures that apparently result from complex processes involving several neurotransmitters namely the glutamatergic, cholinergic, and gabaergic systems [1].Alteration or changes exists in the nature of neuronal networks in the brain which causes seizures and also due to spontaneous expression of synchronized burst firing which interspersed by periods of normal electrical activity [2]. Glutamate and ɣ-amino butyric acid (GABA) are quantitatively the most important excitatory and inhibitory neurotransmitters respectively in the mammalian brain [3]. So these two neurotransmitters are reported as important targets for producing antiepileptic action. Approximately 30% of patients with partial epilepsy and 25% of patients with generalized epilepsy are not completely recovered with allopathic medications[4]. These many patients very of tenly take multiple medical treatments to control their seizures. Thus, there is an unmet need to identify newer molecules with antiepileptic properties. In our study we have chosen herbal medication and it could be one of the sources for newer antiepileptic therapeutics [5]. *Flacourtia indica*(Burm.f.)Merr. Synonymous to Flacourtiara montchi L'Herit.(Family Flacourtiaceae) commonly known as 'Baichi' or 'Katai'. It is an indigenous medicinal plant widely distributed in Bangladesh and India [44].Chufa tubers are daily ingredients of the diet of many people in North Africa and Spain [6]. · Several therapeutically important natural compounds have been isolated (such as alkaloids, flavonoids, carbohydrates, tannins, saponins, and steroids) and they can serve as very potent and reliable drug candidate for treatment of various disorders.This study was undertaken to evaluate the possible anticonvulsant activities of areal parts of *Flacourtia indica* extract using MES and STN induced seizure in rats.

MATERIAL AND METHODS

Plant material

Areal parts of *Flacourtia indica* were collected during December 2018 from, Thirupathi hills, Andhra Pradesh, India. It was identified and authenticated by Prof.Dr. Madhavasetty, Department of Botany, University, Thirupathi, Andhra Pradesh, India. The voucher specimen was maintained in our laboratory for the future reference.

Preparation of extract: Areal parts of *Flacourtia indica* was dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (500gm) of powder was subjected to continuous hot extraction in soxhlet apparatus using ethanol as solvent at a temperature range of 60-70˚C. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample.

Experimental animals: Wistar Albino rats, weighing 150-170g, were procured from the animal house of CES College of pharmacy, Chinnatekur, Kurnool (Reg., no.1278/ac/09/CPCSEA). The animals were kept in polypropylene cages (6 in each cages) under standard laboratory condition (12 hr light and 12 hr dark day night cycle) and had free access to commercial pellet diet with water ad libitum. The temperature was maintained at 25 ± 10C with relative humidity (50 ±15%). The study was approved by the institutional animal ethical committee. Ethical norms were strictly followed during all experiments.

Acute oral toxicity study: The acute toxicity of ethanolic extract of *Flacourtia indica* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). The ethanolic extract of *Flacourtia indica linn* was observed to safe up to 2000mg/kg by oral route. After 24 hours animals were found to be well tolerated. There was no mortality and signs of toxicity. Hence 1/15th(100mg/kg),1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for biological study(IAEC/CESCOP/AUG-2018-03)

Grouping of animals:

In each individual animal model i.e; MES , STN having 4 groups, and each group had six rats. This grouping was common to all 2 animal models. Group I rats received sodium carboxy methyl cellulose (SCMC), Group II received Phenytoin / diazepam, Group III received EEFI 200 mg/kg and Group IV received EEFI 400 mg/kg. In Maximal electro shock seizure (MES) model, Animals exhibit hind limb tonic extension (HLTE) and the percentage of animals protected against HLTE were considered when it is abolished in 10sec and hind limb extension with plane of body.In Strychnine (STN) models**,** Latency of seizure threshold, duration of seizures, % of animals protected against seizures, % of animals protected against lethality were recorded within a thirty minutes duration[9] after intraperitoneal injection of (STN).

Induction of seizures in rats:

1) Maximal electroshock seizure(MES) model :

Test was performed to induce seizures in Albino mice of either sex. Mice were subjected to shock of 150 mA by convulsiometer through ear electrodesfor2seconds on 14th day after 30 minutes of administering the last dose of vehicle, diazepam and extracts. The number of animals exhibiting hind limb tonic extension (HLTE) seizures and the percentage of animals protected against HLTE were recorded [7].

2) Strychnine (STN) model:

Albino rats of either sex were used to induce seizures. On the last day i.e.,14thday,30 min after administration of the last dose of the vehicle, diazepam and the test extracts ,seizures were induced in rats in both models by intraperitoneal injection of Strychnine (STN)-induced seizure with the dose2.5mg/kg. The latency to STN-induced seizures threshold, the duration of seizures, percentage of animals protected against seizures and percentage of animals protected against lethality were recorded within a thirty minutes duration[9] after intraperitoneal injection of (STN) [7].

Statistical analysis:

Data were presented as percentage (%) protection and mean ±SEM and were analyzed by one-way ANOVA followed by Dunnett's test for multiple comparisons using Graph pad prism version 5.03.Results were considered significant at $p<0.05$.

RESULTS

The percentage yield of ethanol extract of entire plant of *Flacourtia indica linn* was found to be 5.8 %w/w respectively.

Acute toxicity study

The results obtained indicated that *Flacourtia indica* extract at oral doses upto 2000 mg/kg did not produce any symptom of acute toxicity and non of the rats died during 72h of observation and up to 14 days. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for biological study.

Table: 1 Phytochemical Constituents

S.No	TEST	INFERENCE
1	Liebermann's test	Steroids absent
2	Salwoski test	Steroids absent
3	Schinoda test	Flavonoids present
4	Ferric chloride test	Tannins present
5	Dragandroff's test	Alkaloids absent
6	Brontanger's test	Anthraquinone absent
7	Kedde's test	Cardinolides absent
8	Legal's test	Cardinolides absent

DISCUSSION

The antiepileptic activity *Flacourtia indica* at two dose levels (200 and 400 mg/kg) was studiedby MES and STN induced seizure models. Antiepileptic drugs which abolish tonic extension occurred by MES acts by inhibiting spread of seizures. Drugs that either prevents or delay seizure occurrence caused by STN , act by elevating the seizure threshold [8]. In our study, in the maximal electro shock seizure (MES) test, 100% of the controlled rats exhibited hind limb tonic extensions (HLTE) seizure. The MES is a standard procedure which evaluates the ability of the testing materials to protect against HLTE. The seizure features in MES are similar for all laboratory animals and human except for the time scale[9].The standard drug diazepam (4mg/kg) and the EECE (200and400mg/kg) exhibited significant anticonvulsant activity and provided protection against electroshock induced HLTE respectively. In the MES, protection against HLTE predicts the anticonvulsant activity of the tested compounds. More over protection against HLTE in MES- induced seizure indicates the efficiency of *Terminalia mollis extract* to either stop or to slowdown the discharge of the seizure within the brain stemsubstrate[10].Seizure induced by MES can be blocked either by inhibiting the voltage-dependent Na^+channels or by blocking glutamatergic excitation mediated by the N-methyl-D-aspartate (NMDA)receptors [11]. Since *Terminalia mollis* extract showed anti-epileptic activity in the MES, it may act by the same mechanism of action [12]. The significant anticonvulsant activities *Terminalia mollis* extract may be due to the presence of many potent compounds or phytoconstituents such as flavonoids, phenols, and terpenes[13]. Strychnine is a neurotoxin which acts as an antagonist of glycogen and acetylcholine receptors. It primarily affects the motor nerve fibers in the spinal cord which control muscle contraction. An impulse is triggered at one end of a nerve cell by the binding of neurotransmitters to the receptors. In the presence of an inhibitory neurotransmitter, such as glycine, a greater quantity of excitatory neurotransmitters must bind to receptors before an action is potentially generated. Glycine acts primarily as an agonist of the glycine receptor, which is a ligand-gated chloride channel in neurons located in the spinal cord and in the brain. The protective effect of the whole plant of *Flacourtia indica* extract against STN-induced convulsions, proposes that it possesses anticonvulsant activity and that glycine neurotransmission is involved. Further phytochemical studies are required to isolate and identify the active molecule(s) responsible for anticonvulsant activity

CONCLUSION

In present study antiepileptic activity of ethanolic extract of *Flacourtia indica* against seizures induced by MES and STN were evaluated. The observed antioxidant and antiepileptic activities are due to the presence of considerable amount of flavonoids and phenolics in the ethanolic extract *Flacourtia indica*. Increased oxidative load is directly implicated as seizures can cause imbalance in oxidant, antioxidant system of brain which leads to oxidation of lipids, DNA and protein ultimately resulting into neurodegeneration. Ethanolic extract of *Flacourtia indica* 400mg/kg was showed good antiepileptic activity in MES as well as STN induced convulsions may be through MES can be blocked either by inhibiting the voltage-dependent Na+ channels or by blocking glutamatergic excitation mediated by the N-methyl-D-aspartate (NMDA) receptors and through glycine inhibitory property compared to 200mg/kg. Thus, results of our study showed promising antiepileptic and anti-oxidant effects of ethanolic extract of Flacourtia *indica* against both the toxicants and provided a scientific claim to the usefulness of this traditional plant in neurological disorders like epilepsy. However, further studies are needed to develop the exact underlying mechanism of antiepileptic action of possible constituents of the plant after isolation of bioactive compound.

Table No 2: EFFECT OF ETHANOLIC EXTRACT *Flacourtia indica (EEFI)* ON MES INDUCED CONVULSIONS IN RATS

Groups	Drug treatment	Tonic Flexion (sec)	Tonic Extensor (sec)	Clonic convulsions (sec)	Stupor (sec)
I	Control	12.17 ± 0.8724###	16.33±2.552###	13.50±2.487###	9.167 ±2.927###
II	Phenytoin 25mg/kg/I.P	2.833 ± 0.6009***	4.333 ± 0.8819***	4.000±0.8563***	3.167± 0.7032 ***
III	*EEFI*200 mg/kg/P.O	6.000±0.8563**	8.167±1.302**	7.000±1.390**	5.833±1.014**
IV	*EEFI*400 mg/kg/P.O	3.500±0.7638***	5.000±0.9661***	5.167±0.9458**	4.000± 05774 ***

Where n=6 the observation are mean±SEM.*P<0.05,**P<0.01and ***P<0.001 as compared to control All the data were analyzed by using one way ANOVA followed by Dunnett'stest. *EEFI*–Ethanolic extract of *Flacourtia indica.*

Table 3: EFFECT OF ETHANOLIC EXTRACT *Flacourtia indica (EEFI)* ON STN INDUCED CONVULSIONS IN RATS

Groups	Drug treatment	Latency (sec)	Onset of Jerky movements (sec)	Onset of Straub's tail (sec)	Onset of Clonic convulsions (sec)	No.of animals alive	% Inhibition
I	STN 2.5 mg/kg/I.P	35.17 ± 4.453###	55.33 ± 5.909###	28.00 ± 3.386###	44.67 ± 5.123###	2	33%
II	Diazepam 4mg/kg/I.P	103.0 ± 11.64***	121.3 ± 13.46***	91.00 ± 9.183***	109.2 ± 8.867***	6	100%
III	*EEFI* 200 mg/kg/P.O	84.17 ± 11.16**	97.83 ± 12.15**	70.17 ± 10.24**	80.83 ± 9.898**	4	66%
IV	*EEFI* 400 mg/kg/P.O	94.50 ± 11.51**	112.0 ± 13.43***	82.83 ± 9.119**	97.67 ± 8.815***	5	83%

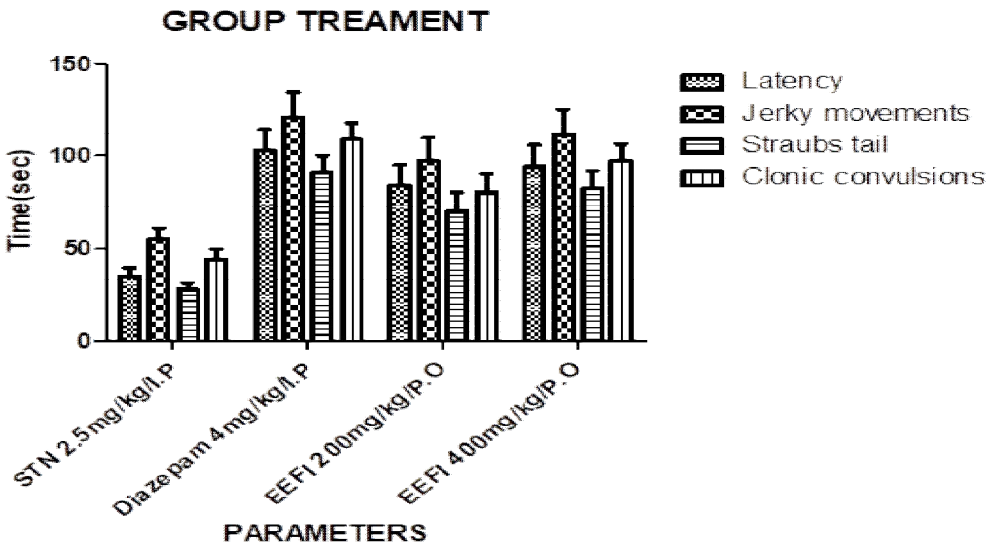
Where n=6 the observation are mean±SEM.*P<0.05,**P<0.01and ***P<0.001 as compared to control All the data were analyzed by using one way ANOVA followed by Dunnett'stest. *EEFI*–Ethanolic extract of *Flacourtia indica.*

Groups	Drug treatment	Lipid peroxidation levels (nm)	Glutathione (nm)	Catalase (nm)	GABA (nm)
I	STN2.5mg/kg/I.P	0.485± 0.02130###	0.1821± 0.008531###	0.2890± 0.01768###	0.6763± 0.01768###
II	Diazepam 4mg/kg/I.P	0.2788 ± 0.01261***	0.4302 ± 0.0009455***	0.7594 ± 0.01498***	0.3722 ± 0.02098***
III	*EEFI* 200 mg/kg/P.O	0.3082 ± 0.01370**	0.3288 ± 0.01429**	0.5848 ± 0.01291**	0.4032 ± 0.01392**
IV	*EEFI*400 mg/kg/ P.O	0.2558 ± 0.01197***	0.3792 ± 0.01726***	0.6954 ± 0.01788***	0.3457 ± 0.01654***

Table4: Effectof Ethanolic Extract *Flacourtia Indica (Eefi)* On STN Induced Convulsions in Rats - Antioxidant Studies

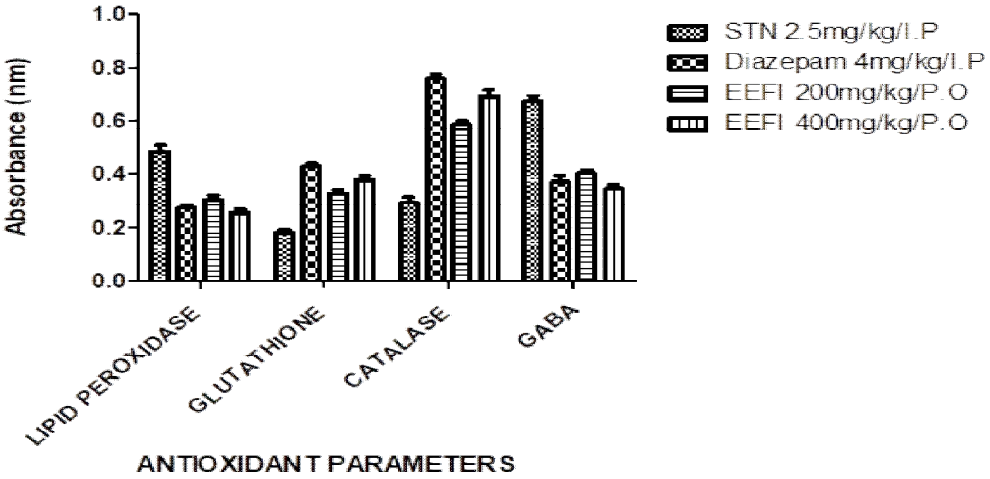
Where n=6 the observation are Mean±SEM.*P<0.05,**P<0.01and ***P<0.001 as compared to control All the data were analyzed by using one way ANOVA followed by Dunnett's test.
EEFI–Ethanolic extract of *Flacourtia indica.*

Figure 1: Effect of *EEFI* on Strychnine induced convulsions in rats



Where n=6 the observation are Mean ± SEM. *P<0.05, **P<0.01 and ***P<0.001 in comparison to the control all of the data was evaluated with a one-way ANOVA and Dunnett's test.
***EEFI**:* Ethanolic extract of *Flacourtia indica*.

Figure 2: : **Effect of *EEFI* on Strychnine induced convulsions in rats antioxidants levels**



Where n=6 the observation are Mean ± SEM. *P<0.05, **P<0.01 and ***P<0.001 in comparison to the control all of the data was evaluated with a one-way ANOVA and Dunnett's test.
***EEFI**:* Ethanolic extract of *Flacourtia indica*.

Acknowledgment: This work was supported by Creative Educational Society's College of Pharmacy, Chinnatekur, Kurnool, and Andhra Pradesh, India.

Conflict of interest: The authors reports no conflicts of interest

Funding: Self funded

Author's Contribution: All the authors are actively contributed entire work

Ethics Statement: IAEC/CESCOP/AUG-2018-03

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CITATION OF THIS ARTICLE

Chakali Ayyanna, T. Manoj Kumar , Md. Althamas, *D.Vamshi Krishna,* Supraja Koneti. Evaluation Of Anticonvulsant Propertiy Of *Flacourtia Indica*in Mes And STN Induced Seizures In Rats. Bull. Env. Pharmacol. Life Sci., Vol 12[6] May 2023: 61-66.