EVALUATION OF ANTICONVULSANT PROPERTY OF *Senna singueana* ETHANOLIC ROOT EXTRACT IN MAXIMAL ELECTRO SHOCK AND STRYCHNINE INDUCED CONVULSIONS IN RATS

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ABSTRACT

Epilepsy disease continues to be a neurological disease, and safer drugs with improved anticonvulsant efficacy are awaited.

The purpose: In the present study, the ethanolic extract of *Senna singueana* root was investigated for protective effects against maximal electric shock (MES) and strychnine (STN)-induced seizures in rats.

Method: Anti-convulsant animal models included maximal electric shock (MES) and strychnine (STN). Each animal model (MES, STN) comprised four groups of albino rats (n=6). First is control, second standard, third and fourth tested MES generated seizures with a 60 Hz alternating current at 150 mA for 0.2 s. In the third and fourth groups, rats were pre-treated with an ethanolic extract of *Senna singueana* root (EESS) 200 and 400 mg/kg/p.o. for 14 days, while the standard group was pre-treated with phenytoin (25 mg/kg/i.p.). For strychnine-induced convulsions, animals in the third and fourth groups were pre-treated with EESS 200 and 400 (mg/kg/p.o) for 14 days, whereas those in the standard group received diazepam (4 mg/kg/ip). Strychnine (2.5 mg/kg/i.p.) caused convulsions on day 14.

Results: Ethanolic extract of senna singueana root (EESS) reduced the duration of hind limb tonic extension (HLTE) stage in MES-induced epilepsy in a dose-dependent manner and showed more activity at 400 mg/kg compared to standard group animals. Seizure protection was 75% and 100% with 200 and 400 mg/kg/p.o. In STN-induced convulsion paradigm, 200 and 400 mg/kg EESS delayed the onset of Latency phase and prevented death in 66% and 83% of rats, respectively, compared to the standard group.

Conclusion: Ethanolic extract of *Senna singueana* root showed potent and dose-dependent antiepileptic action, probably due to antioxidant principles such flavanoids, phenolics, and other phytoconstituents that protect against MES and STN models.

Key Words: Anti epileptic activity, *Senna singueana*, Maximal electric shock, Strychnine, Phenytoin, Diazepam

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INTRODUCTION:

Recurrent spontaneous convulsions in people with epilepsy are thought to originate from a wide range of neurotransmitter systems, including glutamatergic, cholinergic, and gabaergic systems[1]. Epileptic seizures are characterized by synchronous firing bursts followed by intervals of normal electrical activity[2], which occur as a result of a change in the structure or function of neural networks in the brain. The two most abundant neurotransmitters in the mammalian brain are glutamate and gamma-aminobutyric acid (GABA), respectively [3]. As a result, both of these neurotransmitters are receiving attention as potential sites for producing antiepileptic effects. Patients with partial-onset seizures are less likely to totally recover from epilepsy than those with generalized epilepsy (around 30% vs. 25% respectively) when treated with symptomatic medications[4]. Multiple treatments are generally tried by these patients before the seizures are under control. The search for novel compounds with anti-epileptic effects is thus recommended. The study we conducted focused on African herbal remedies since they have the potential to be used in the development of new antiepileptic drugs[5].

Other names for *Senna singueana* include Cassia sabak (Delile), Cassia sennae (Fresen), Cassia kethulleana (DeWild), and Cassia singueana (Delile). Comes from the family fabaceae. *Senna singueana* is a widespread plant in dry or somewhat dry climates. Several countries in Africa can claim this plant as their own. *Senna singueana*, a plant with therapeutic potential, has long been utilized in folk medicine throughout a wide swath of Africa. *Senna singueana* has a variety of pharmacological characteristics, including antibacterial, antimalarial, antifungal, antidiabetic, and antioxidant activities [6], which can be found in the powdered leaves, stems, roots, flowers, and bark. However, no reports were available to back up its ethnobotanical claim until now. Therefore, the objective of this study was to determine whether or not an ethanolic root extract of *Senna singueana* could inhibit the maximal electroshock (MES) and Strychnine (STN)-induced convulsions.

Materials and methods

Collection and authentication of plant:

Roots of *Senna singueana* were collected during December 2022 from the hills of thirupathi, 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: Roots of *Senna singueana* 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.

Pharmacological Investigation:

Experimental animals: Rats of both sexes weighing 150-200g were obtained from the approved CPCSEA (Reg no:1278/ac/09/CPSEA) Animal Facility of CES College of Pharmacy, Chinnatekur, Kurnool,A.P. Animals were maintained under standard laboratory conditions with 12:12 h light/dark cycle in a polypropylene cage(6 in each cages). Animals were fed a standard pelleted diet with water *ad libitum*. The experimental procedures were approved by the institute's Institutional Animal Ethics Committee (IAEC) (CPCSEA /IAEC/CESCOP/2023-07) and experiments were conducted strictly according to the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animal (CPCSEA).

Acute oral toxicity study: The acute toxicity of ethanolic extract of *Senna singueana* root was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). The ethanolic root extract of *Senna singueana* 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 $1/15^{th}$ (100mg/kg), $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected for biological study (IAEC/CESCOP/2023-07). Effects such as changes in skin fur, eyes and mucous membranes were observed daily. Animals were further observed for salivation, diarrhoea, tremors, lethargy, convulsions, sleep and coma. Parameters such as body weight, food intake and water intake were checked every two days[7].

Evaluation of antiepileptic activity:

MES –induced convulsions in rats: In this method, a group of 24 rats (170-200 g) was used. Divided into Four different groups of six rats each.

1) Group I - Served as a control (received normal saline).
2) Group II - Received Phenytoin (25mg/kg i.p)

served as a standard.
3) Group III - Received *EESS* (200mg/kg p.o) served as a test-I.
4) Group VI - Received *EESS* (400mg/kg p.o) served as a test-II.

Rats 150-200 g animals of both sexes (n=6 in each group) were used for the study. All animals were respectively treated for 14 days prior to convulsion. On day 14, all groups were induced with an electroconvulsive meter. The maximum electric shock convulsions are generated by a current of 150 mA, 60 Hz for 0.2 s [8]. Put a drop of electrolyte solution (NaCl 0.9%) on the corneal electrodes before administering to rats, this increases exposure and reduces mortality. Duration of various phases (such as flexion, extension, tonic convulsions, stupor, and recovery or death) of epilepsy has been observed. The protection rate is estimated by observing the number of animals showing abolition and duration of Hind Limb Tonic Extension (HLTE).

STN –induced convulsions in rats:

1) Group I -Received STN 2.5mg/kg/I. P served as control.
2) Group II - Received Diazepam (4mg/kg i.p) served as standard.
3) Group III - Received *EESS* (200mg/kg p.o) served as a test- I.
4) Group IV - Received *EESS* (400mg/kg p.o) served as a test-II.

Rats 150-200 g animals of both sexes (n=6 in each group) were used for the study. The experimental groups (III and IV) received 200 mg/kg, 400 mg/kg of EESS orally for 14 days, respectively, and testing was performed for antiepileptic activity 1 hour after the last dose of the extract. Strychnine (2.5 mg/kg) was used as an inducer[9]. After strychnine injection, the animals were placed in an individual plastic cage to observe seizures. Standard group animals received diazepam (4.0mg/kg/i.p) on 14th day 1hr prior to Strychnine administration[10].

Statistical analysis:

Graph Pad Prism 6.0 software was used in the statistical analysis of the experimental data. Data were presented as percentage (%) protection and mean ±SEM and were analyzed by one-way ANOVA followed by Dunnet's multiple comparative tests. $p< 0.001$, $p< 0.01$ and $p< 0.05$ is considered as significant.

RESULTS AND DISCUSSION

The percentage yield of ethanolic root extract of *Senna singueana* was found to be 4 %w/w respectively.

Table: 1 Phytochemical Constituents:

S.NO	TEST	INFERENCE
1	Dragendroff's test	Alkaloid's present
2	Lieberman's test	Phytosterols present
3	Salkowski test	Phytosterols present
4	Tannin's test	Tannin's present
5	Shinoda test	Flavonoid's present
6	Biuret test	Proteins absent
7	Anthocyanin's test	Anthocyanin's present
8	Quinones's test	Quinine's present
9	Phenolic test	Phenol's present

Acute toxicity study:

The results obtained showed that oral administration of *Senna singueana* extract up to 2000 mg/kg did not show any symptoms of acute toxicity, and no rats died during the 72 hours of observation and up to 14 days of observation was shown. Therefore, 1/10 (200 mg/kg) and 1/5 (400 mg/kg) of this dose were selected for biological studies.

Table 2: EFFECT OF *EESS* ON MES INDUCED CONVULSIONS IN RATS

Groups	Drug treatment	Tonic Flexion (sec)	Tonic Extensor (sec)	Clonic Convulsion (sec)	Stupor (sec)	Recovery or Death
I	Control	10.52 ± 0.5400$^{###}$	15.67 ±0.7149$^{###}$	13.50 ±0.7638$^{###}$	18.50 ±1.025$^{###}$	6
II	Phenytoin 25 mg/kg/I. P	3.00 ±0.4830***	1.00±0.4830***	4.00 ±0.4626***	4.20 ±0.7832***	6
III	*EESS* 200 mg/kg/P. O	6.5 ±0.9661**	3.00±0.5332**	7.00 ±0.6952**	8.50 ±0.7638**	6
IV	*EESS* 400 mg/kg/ P. O	4 .00±0.5797***	1.500±0.4575***	5.00 ±0.6132***	5.75 ±0.8921***	6

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

Table 3: EFFECT OF *EESS* ON MES INDUCED CONVULSIONS -ANTIOXIDANT STUDIES

Groups	Drug treatment	Lipid Peroxidation levels(nm)	Catalase levels(nm)	Glutathione levels(nm)	SOD Levels(nm)	GABA levels(nm)
I	Control	0.5223±0.02257$^{###}$	0.2950±0.03897$^{###}$	0.4080±0.04420$^{###}$	0.3060±0.02552$^{###}$	0.1280±0.01313$^{###}$
II	Phenytoin 25 mg/kg/I. P	0.2887±0.03874***	0.6445±0.02645***	0.7650±0.03294***	0.7150±0.03170***	0.3017±0.02007***
III	*EESS* 200 mg/kg/P. O	0.3670±0.02825**	0.4730±0.02686**	0.5817±0.03114**	0.4267±0.04137**	0.1738±0.0970**
IV	*EESS* 400 mg/kg/P. O	0.3203±0.02944***	0.6100±0.02733***	0.7000±0.02933***	0.6450±0.02566***	0.2655±0.01374***

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

Table 4: EFFECT OF *EESS* ON STRYCHNINE INDUCED CONVULSIONS IN RATS

Groups	Drug treatment	Onset of Latency(sec)	Onset of Jerky movements(sec)	Onset of Straub's tail (sec)	Onset of Clonic convulsions (sec)	No. ofanimals alive	%Inhibition
I	STN 2.5 mg/kg/I. P	65.00±6.583$^{###}$	68.17±3.87 $^{###}$	27.67±1.542$^{###}$	40.00±2.556$^{###}$	2	33%
II	Diazepam 4mg/kg/I. P	180.00±17.510***	34.00±1.983***	62.17±2.272***	17.00±0.966***	6	100%
III	*EESS* 200 mg/kg/P. O	120.70±8.212**	52.67±2.813**	41.17±2.088**	28.67±1.282**	4	66%
IV	*EESS* 400 mg/kg/ P. O	170.20±12.090***	40.17±3.719***	55.33±1.856***	20.83±1.579***	5	83%

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. *EESS* – Ethanolic extract of *Senna singueana* root.

Table 5 : EFFECT OF *EESS* ON STRYCHNINE INDUCED CONVULSIONS –ANTIOXIDANT STUDIES

Groups	Drug treatment	Lipid Peroxidation levels(nm)	Glutathione levels(nm)	Catalase levels(nm)	SOD Levels(nm)	GABA levels(nm)
I	STN 2.5 mg/kg/I.P	0.4358±0.01540$^{###}$	0.2800±0.01673$^{###}$	0.4400±0.02840$^{###}$	0.3500±0.02989$^{###}$	0.4800±0.0293$^{###}$
II	Diazepam4mg/kg/I. P	0.2042±0.01471***	0.5612±0.02634***	0.8435±0.01895***	0.8100±0.03296***	0.7367±0.02667***
III	*EESS* 200mg/kg/P. O	0.3225±0.01601**	0.4100±0.02933**	0.5000±0.02989**	0.4450±0.03631**	0.5670±0.04410**
IV	*EESS* 400 mg/kg/P. O	0.2107±0.02795***	0.5000±0.02556***	0.7433±0.02801***	0.6500±0.02989***	0.7017±0.03842***

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. *EESS* – Ethanolic extract of *Senna singueana root.*

DISCUSSION:

MES and STN-induced seizure models investigated the antiepileptic effects of *Senna singueana* at 200 and 400 mg/kg. Antiepileptic reduces MES tonic extension by preventing seizures. STN-preventive drugs increase the seizure threshold [11]

In the maximal electrical shock seizure (MES) test, all controlled rats had hind limb tonic extensions (HLTE) seizures. The MES tests substances for HLTE protection. Except for time scale, all laboratory animals and humans have MES seizures[12].Diazepam (4mg/kg) and EESS (200–400mg/kg) had considerable anticonvulsant action and protected against electroshock-induced HLTE. MES HLTE protection predicts anticonvulsant action. *Terminalia mollis* extract can block or decrease seizure discharge in the brain stem substrate, protecting against HLTE in MES-induced seizures[13].Inhibiting voltage-dependent Na+channels or NMDA receptor-mediated glutamatergic excitement can prevent MES-induced seizures[14]. *Terminalia mollis* extract's MES anti-epileptic efficacy suggests a similar mechanism[15]. Flavonoids, phenols, and terpenes in *Terminalia mollis* extract may contribute to its anticonvulsant properties[16].

Phenytoin (PHT)-treated animals showed 100% protection against MES-induced seizures, while 200 and 400 mg/kg EESS showed protection, respectively 100 % and 100% against seizures due to MES (Table No:2). EESS at both 200 and 400 mg/kg doses showed significant antiepileptic activity ($p<0.05$ and $p<0.01$) compared with control (Table No:2).

Neurotoxin strychnine blocks glycogen and acetylcholine receptors. It primarily impacts spinal cord motor nerve fibers that contract muscle. Neurotransmitters and receptors initiate nerve cell impulses. Inhibitory neurotransmitters like glycine require more excitatory neurotransmitters to bind to receptors before an action may occur. Glycine is predominantly an agonist of the glycine receptor, a ligand-gated chloride channel in spinal cord and brain neurons.

Animals treated with diazepam showed 100% protection against strychnine-induced seizures, whereas EESS 200 and 400 mg/kg provided 66% and 83% protection against strychnine-induced seizures(Table No:5), respectively showed. EESS showed significant ($p<0.05$ and $p<0.01$) antiepileptic activity at both 200 mg/kg and 400 mg/kg doses compared to control (Table No:5).

Senna singueana ethanolic root extract protects against STN-induced convulsions, suggesting anticonvulsant efficacy and glycine neurotransmission. Further phytochemical studies are required to isolate and identify the active molecule(s) responsible for anticonvulsant activity.

Conclusion:

MES and STN-induced seizures were tested for antiepileptic efficacy of *Senna singueana* ethanolic extract. Flavonoids and phenolics in *Senna singueana* ethanolic extract cause its antioxidant and antiepileptic effects. Seizures can produce brain oxidant-antioxidant imbalance, oxidizing lipids, DNA, and protein causing neurodegeneration. Ethanolic *Senna singueana* 400mg/kg demonstrated good antiepileptic efficacy in MES and STN. MES-induced convulsions can be prevented by suppressing voltage-dependent Na+ channels, NMDA receptors, or glycine compared to 200mg/kg. Thus, ethanolic extract of *Senna singueana* exhibited promising antiepileptic and antioxidant activities against both toxicants and supported the use of this traditional plant in neurological illnesses like epilepsy. After bioactive chemical isolation, more research is needed to find out the plant's constituents' antiepileptic mechanism.

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Conflict of interest: The authors reports no conflicts of interest

Ethics Statement: IAEC/CESCOP/2023-07.

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