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ANTICONVULSANT EFFECT OF ETHANOLIC EXTRACT OF *Senna singueana* (DEL). LOCK ON PENTYLENE TETRAZOLE (PTZ) INDUCED CONVULSIONS IN MICE

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ABSTRACT

The study was undertaken evaluate the anticonvulsant activity of *Senna singueana* in pentylenetetrazole (PTZ) induced seizure model in mice**.** In this model, 5 groups of albino mice (n=6) were used. The 1st group was kept as control, 2nd as standard (diazepam, 4 mg/kg); 3rd - 5th were treated with *Senna singueana* ethanol root extract (100, 200 and 400 mg/kg). After 30 min of administration, the mice were I.P injection of PTZ (75 mg/kg). Anticonvulsant activity was confirmed by measuring the latency to PTZ -induced threshold seizures, and the duration of seizures in the mice. Extract at 400 mg/kg prolonged latency to seizure onset in PTZ model respectively**.** *Senna singueana* possess anticonvulsant effect by delay the latency of seizures produced by PTZ.

Keywords: *Senna singueana*, Anticonvulsant, Diazepam, Pentylenetetrazole,

INTRODUCTION

Epilepsy is a neurological disease which affects around sixty-five million people worldwide **[1]**. It can be defined as a chronic and dynamic neurological condition of an abnormal, excessive, hyper synchronous discharge of a population of cortical neurons **[2]** associated with ongoing neuronal damage, particularly when uncontrolled and causes physical, psychological, and social abnormalities in patients. Epilepsy posses very complex pathophysiology involving neuronal plasticity, imbalance between gama-aminobutyric acidergic (GABAergic) inhibitory and gluatamatergic excitatory neurotransmitter system and ion exchange dysfunction. Thus a decrease in concentration of gama-amino butyric acid (GABA) leads to many pathological processes in the brain which may be manifests in to convulsive episodes **[3]**. Recurrent spontaneous seizures may enhance reactive oxygen species (ROS) in the central nervous system **[4]**. In about 70 %cases, there is no understandable cause. This type of epilepsy often arises is in childhood **[5]**, with a high prevalence of about 0.8% below the age of 7 y **[6]**. A significant number of drugs are accessible for the treatment of different types of seizures. The aim is to decrease the incidence and severity of seizures within a scaffold of a suitable level of side effects. A perfect antiepileptic drug would restrain all seizures without causing any untoward consequence, but unluckily the drugs that are used currently not only fail to suppress seizure activity in some patients, they also produce recurrent adverse effects **[7]**.The copious side effect profile of the currently available antiepileptic drugs (AEDS) are of utmost distress for both the patients and their physicians. Numerous efforts have been made previously to get hold of anticonvulsive drugs from plant sources and these attempts will continue until a suitable treatment option is obtainable **[8]**. Natural products from folk remedies have made significant contributions in the breakthrough innovation of modern drugs and can serve as an alternative option for the discovery of AEDs with novel structures and better tolerance **[9]**.

Senna singueana (Del.) Lock (Syn: *Cassia singueana*; legume) has many medical uses in Africa **[10]**, including traditional use in the fight against malaria. In Tanzania, its leaf juice was drunk to combat malaria **[11]**, and in Kenya **[12]** and Burkina Faso **[12, 13]** it was noted that warm powdered leaf soup taken by mouth can prevent malaria and disease fever **[11, 13]**. Previous studies have shown that methanol plant root bark extract has significant anti-Plasmodium activity against Plasmodium berghei **[14]**. The root bark also reportedly contains lupeol **[10]** atriterpene, which has a wide range of biological activities, including antimalarial effects in P. falciparum **[15]**. Although *S. Singueana* has many medical uses, there are few studies on its pharmacology and it is limited to the root bark. Due to its multiple medicinal uses, it is necessary to study the characteristics of its leaves because they are more sustainable than root bark or stem bark. Furthermore, scientific claims have shown that various leaf extracts have antioxidant activity in vitro and in vivo **[16-18]**. This study used the anticonvulsant activity of EESS in mice induced by pentylenetetrazole (PTZ).

MATERIALS AND METHODS:

Drugs and chemicals:

Plant material: Roots of *Senna singueana* roots were collected during December 2019 from, Thirupathi hills, Andhra Pradesh, India. It was identified and authenticated by Prof.Dr. Madhavasetty, Botanist, University, Thirupathi, Andhra Pradesh, India. The voucher specimen was maintained in our laboratory for the future reference

Preparation of plant extract: Roots of was dried in shade separated and made to dry powder. It was then passed through the 40 mesh sieve. Sufficient quantity 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. Yield thick green semisolid residue (EESS, 5.8% w/w).

Experimental Animals: Albino mice, weighing 25-30g, 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 cage) under standard laboratory conditions (12 hours of light and 12 hours of day-night dark cycle), the animals are housed in polypropylene cages (6 per cage) and have free access to commercial pellets and free drinking water. The temperature is kept at 25 ± 10 °C and the relative humidity (50 ± 15%). The study was approved by the Institutional Animal Ethics Committee (IAEC / CESCOP / AUG-2018-03). All experiments are strictly governed by ethical standards.

Acute oral toxicity studies: According to OECD guidelines No.423 (Acute toxicity classification method), the acute toxicity of the ethanolic root extract of *Senna singueana* was determined. *Senna singueana* ethanolic root extract was observed to safe up to 2000mg/kg by oral route. After 24 hours, the animals were found to be well tolerated. There are no signs of mortality or toxicity. Therefore, choose 1/15 (100 mg / kg), 1/10 (200 mg / kg), and 1/5 (400 mg / kg) of this dose for biological research **[8]**.

Methods:

Pentylenetetrazole (PTZ) seizure model:

The mice of 25-30g of either sex animals (n=6 in each group) were used for the study. The test groups (III, IV& V) received *Senna singueana* ethanol extract

(100mg/kg) , *Senna singueana* extract (200mg/kg) & *Senna singueana* extract(400mg/kg) orally for 14days. Respectively and test conducted for antiepileptic activity 1hr after the last dose of the extract. PTZ (75mg/kg) is used as the inducing agent. After the administration of PTZ, the animals were placed in an individual polypropelene cage for observation convulsions. Standard group animals were received Diazepam (4mg/kg) on 14th day 1hr prior to PTZ administration. There are four distinct phases constituted the PTZ (75mg/kg i.p) seizure sequence i.e. Latency, Jerky movements, Straub's tail, clonic convulsions & Death/Recovery.

Statistical Analysis:

Data were expressed 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 $p < 0.05$. (Prism graph pad 5.3 version).

RESULTS AND DISCUSSION

The percentage yield of ethanol extract of entire plant of *Senna singueana* was found to be 2.94%w/w respectively.

Acute oral toxicity studies:

The results obtained indicated that *Senna singueana* ethanolic root extract at oral doses up to 2000 mg/kg did not produce any symptom of acute toxicity and none of the rats died during 72 hr of observation and up to 14 days.

Table 1: Summary of 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

ANTICONVULSANT ACTIVITY

Table 2: Effect of EESS on PTZ Induced Convulsionsins in Mice

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	Control (PTZ 75mg/kg/i. p)	29.83±8.86	60.5±6.791	21.5±6.791	39.83±8.86	2	33%
II	Diazepam (4mg/kg/i. p)	160.5±9.868***	165.5±10.93***	103.2±9.857***	170.5±9.868***	6	100%
III	*EESS* (100mg/kg/p. o)	110.0±9.747*	107.7±12.53*	64.67±12.72*	120.0±9.747*	4	66%
IV	*EESS* (200mg/kg/p. o)	130.2±18.5**	131±13.48**	80.3±13.55**	160.2±18.50**	5	83%
V	*EESS* (400mg/kg/p. o)	210±32.76***	145.2±14.40***	97.17±12.72***	220±32.76***	6	100%

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

Table 3: PTZ Induced Convulsions- Antioxidant Studies

Groups	Drug treatment	LPO (nm)	Glutathione levels(nm)	SOD levels(nm)	Catalase (nm)	GABA levels(nm)
I	PTZ (75mg/kg)	0.8000±0.0493	0.800±0.040	0.1413 ±0.07090	0.2637±0.08020	0.1413 ±0.07099
II	Diazepam (4mg/kg)	1.504±0.08915***	1.6.33±0.0054***	0.6567 ±0.02883***	0.8067±0.02883***	0.6567 ±0.02883***
III	*EESS*(100mg/kg)	0.5297±0.0464*	0.606±0.059*	0.3833±0.07902*	0.5042±0.09177*	0.3833 ± 0.07902*
IV	*EESS*(200mg/kg)	0.5087±0.0464**	1.037±0.042**	0.4233±0.04208**	0.6167±0.03730**	0.4233 ±0.4208**
V	*EESS*(400mg/kg)	0.1677±0.042***	1.375±0.049***	0.8950±0.03344***	1.050±0.03430***	0.8950 ±0.03344***

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

DISCUSSION

The results of this study demonstrate that the ethanolic root extract of *Senna singueana* has anticonvulsant activity. EESS doses of 100, 200, and 400 mg/kg significantly delayed the onset of seizures, significantly reducing seizure duration in mice resistant to pentylenetetrazole-induced seizures. The standard antiepileptic drug, diazepam (4 mg/kg), completely antagonizes pentylenetetrazole-induced seizures. PTZ can induce convulsive effects by inhibiting GABA activity at GABA-A receptors **[19]**. GABA is a major inhibitory neurotransmitter in the brain, and its inhibition of neurotransmitters is thought to be an underlying factor in epilepsy **[20]**. Improvements in GABAergic neurotransmission are reported to counteract seizures, while inhibition of seizure-promoting neurotransmitters **[21]**. It is expected that protection of rats from PTZ-induced seizures with standard anticonvulsants, phenobarbital, and diazepam as multiple authors have shown that they exert an anticonvulsant effect by enhancing GABA-mediated inhibition **[22]**.

Therefore, the results of this study tend to suggest that EESS with anticonvulsant activity may have inhibited and / or attenuated PTZ-induced seizures in mice given the drug used by increasing or interfering with a somehow with allergic GABA neurotransmission. Doses of 100, 200 and 400 mg / kg of EESS significantly delayed onset of seizures and significantly reduced seizure duration in mice versus PTZ-induced seizures **(Table 2)**. A dose of EESS of 400 mg / kg showed 83.33% protection in mice against seizures caused by PTZ **(Table 2)**. The standard anticonvulsant, diazepam 4 mg / kg i.p. completely eliminated the effects of PTZ-induced seizures in mice **(Table 2)**. It is well known that one of the factors that cause PTZ-induced seizures is a subcritical decrease in GABA in some cells. PTZ has been shown to reduce GABA levels in the brain to comparable levels in rats and mice **[23]**. Perhaps a decrease in GABA stores could lead to a decrease in the amount of GABA released by nerve impulses. Thus, GABA receptors are up regulated to a maximum degree of sensitivity to maximize GABA activity. Diazepam showed a marked protective effect against PTZ-induced seizures in mice **[24]**. PTZ-induced seizures in mice significantly delayed the onset of seizures, reducing the duration of action. The results showed that EESS showed anticonvulsant activity against PTZ-induced seizure patterns.

Free radicals have been suggested to be the most likely candidates responsible for inducing neural changes that mediate behavioural deficits in neurons. Antioxidants are effective in rodent models of epilepsy, stroke, and Alzheimer's disease **[25]**. Therefore, the effect of EESS on oxidative stress in PTZ-induced seizures was also evaluated. The mice pretreated with EESS doses of 100, 200 and 400 mg/kg showed a significant reduction in the levels of malondialdehyde in the brains of the rats compared with the control group **(Table 3)**. Glutathione is an endogenous form of antioxidant in free radicals and prevents the generation of hydroxyl radicals, the most toxic form of free radicals **[25]**. The reduced levels of glutathione in the control mice observed in the present study **(Table 3)** suggest that there is an increase in the generation of free radicals and the reduced amount of glutathione which was depleted during the fight against oxidative stress **[26]**. EESS 100,200 and a dose of 400 mg / kg showed significantly increased levels of GSH in rat brain (Table 3). Decreased MDA and increased glutathione levels in EESS + PTZ mice suggest that EESS has a good antioxidant effect. Several studies have also shown that antioxidant activities on medicinal plants are due to the presence of polyphenols and flavonoids **[27]**.

Phytochemical studies indicate the presence of phenolic and flavonoid compounds in the EESS. Various phytochemicals are thought to have activities on the CNS **(Table 1)**. Anticonvulsant activity is attributed to alkaloids **[28]**, essential oils **[29]**, flavonoids (30), triterpene steroids and triterpenoid saponins which are believed to have anticonvulsant activity in several experimental epilepsy models such as MES and PTZ **[31, 32]**. It has also been found that many flavonoids can act as benzodiazepine-like molecules in the central nervous system and modulate GABA-induced chloride flux in animal models of anxiety, sedation, and anxiety. In the present study, the anticonvulsant activity could be attributed to the presence of alkaloids, steroids, flavonoids, tannins and saponins in the EESS.

CONCLUSION

In this study, the protective effect of ethanolic root extract of *Senna singueana* against seizures induced by PTZ was evaluated. The observed antiepileptic activity is due to a significant amount of flavonoids and phenolics in the ethanolic root extract of *Senna singueana.* 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 root extract of 400mg/kg *Senna singueana* showed good antiepileptic activity in PTZ induced convulsions may be through glycine inhibitory property compared to 100mg/kg & 200mg/kg. Therefore, the results of our study show the promising antiepileptic effect of ethanolic root extract of *Senna singueana* 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 constituents.

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