ISSN: 0976-4607
Research Article

International Journal of Innovative Pharmaceutical Research

Journal homepage: www.ijipr.com

Anti Epileptic Activity of *Enicostema Axillare* against MES and PTZ Induced Seizures in Rats

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ABSTRACT

The present report is an investigation of anti-seizure activity of *Enicostema axillare* is a well known plant which is used in Indian traditional medicine for Diabetes mellitus, Rheumatism, Abdominal ulcers, Hernia, Inflammation, Epilepsy, Cancer and Hepatic disorders. The aqueous and Chloroform extracts of *Enicostema axillare* were subjected to acute toxicity studies and then screened for antiepileptic activity by maxima electroshock (MES) and pentylenetetrazole(PTZ) induced seizures in albino wistar rats . Acute toxicity studies of extracts were nontoxic up to recommended dose 2000mg/kg body weight orally as per OECD guidelines no.423. Animals were treated with aqueous extract of 200,400 mg/kg body weight and chloroform extract of 200,400 mg/kg body weight. The study in Pentylenetetrazole induced seizure test, onset of myoclonic spasm and clonic convulsions were delayed in test groups. Aqueous and Chloroform extracts of *Enicostema axillare* showed anticonvulsant activity against MES and PTZ animal models. Chloroform extract has showed better activity than aqueous form.

Keywords: Epilepsy, *Enicostema axillare,* MES, PTZ.

INTRODUCTION

Epilepsy (Greek - to seize) is a common chronic neurological disorder characterized by seizures (Blume *et al*., 2001). These seizures are transient signs and/or symptoms of abnormal, excessive or synchronous neuronal activity in the brain (Fisher *et al*., 2005). People have seizures when the electrical signals in the brain misfire. The brain's normal electrical activity is disrupted by these overactive electrical discharges, causing a temporary communication problem between nerve cells.

About 50 million people worldwide have epilepsy, and nearly two out of every three new cases are discovered in developing countries (WHO, 2011). Epilepsy is more likely to occur in young children or people over the age of 65 years, however, it can occur at any time. As a consequence of brain surgery, epileptic seizures may occur in recovering patients (National society for epilepsy, 2009).

Epilepsy is usually controlled, but cannot be cured with medication, although surgery may be considered in difficult cases.

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However, over 30% of people with epilepsy do not have seizure control even with the best available medications (Cascino, 1994; Engel, 1996). Not all epilepsy syndromes are lifelong some forms are confined to particular stages of childhood. Epilepsy should not be understood as a single disorder, but rather as syndromic with vastly divergent symptoms but all involving episodic abnormal electrical activity in the brain.

Enicostema axillare non (Blume) A. Raynal is commonly known as Indian Whitehead belonging to the family Gentianaceae. The plant is native to tropical Africa, India, Southeast Asia and Malaysia; africa to Lesser Sunda Islands, It is a perennial herb found throughout India and is common in coastal areas. The plant is used in folk medicine to treat, Diabetes mellitus, Rheumatism, Abdominal ulcers, Hernia, Inflammation, Epilepsy, Cancer, Hepatic disorders (Amritpal singh, 2008).

MATERIALS AND METHODS

Plant collection

The whole plant of *Enicostema axillare* non (Blume) A. Raynal, was collected from Tirupati, Andhra Pradesh, India. It was authenticated by Dr.K.Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati.

Preparation of Extracts

The plant material was collected and dried under shade. Dried material was blended to powder using a blender. The dried powder was sieved to fine powder for extraction with chloroform and water.

Methods

1. Maceration (For aqueous extract)
2. Continuous hot percolation (For chloroform extract)

PRELIMINARY PHYTOCHEMICAL SCREENING

The phytochemical examination of aqueous and chloroform extract of *Enicostema axillare* non (Blume) A. Raynal was performed by standard methods (Harbone, 1973).

ANIMALS USED

Inbred albino rats (150-200gm) of either sex were obtained from the animal house in Sree Vidyaniketan College of Pharmacy, Tirupati. The rats were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages.

ACUTE TOXICITY STUDY

Toxicity is the degree to which a substance is able to damage an exposed organism. In screening drugs, determination of LD_{50} (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals) is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance. It is an initial assessment of toxic manifestations (provides information on health hazards likely to arise from short-term exposure to drugs) and is one of the initial screening experiments performed with all compounds

ANTI EPILEPTIC ACTIVITY

Effect on Maximal electroshock (MES) induced seizures

Albino rats of 150-200 g body weight were divided into three groups of six animals each. The first group, which received saline orally, served as the control whilst the second group received 30 mg/kg of phenobarbitone sodium intraperitoneal (i.p), and third group received 50 mg/kg of BM (p.o). After an hour of treatment, convulsions were produced in rats using an "Inco" convulsiometer by delivering current of 150 mA through corneal electrodes for a period of 0.2 seconds. The severity of convulsions was assessed by the duration of flexion, extension, clonus, stupor and recovery phase for each animal. Inhibition of extensor phase was studied in this model.

Pentylenetetrazole-Induced Convulsions in Rats

Albino rats of 150-200 g body weight were divided into five groups of six animals each. The first group, receiving saline orally, served as control whereas the second group received 4mg/kg of diazepam intraperitoneally (i.p); the third group received a sub protective dose of diazepam, i.e. 0.5 mg/kg i.p.; the fourth received 50 mg/kg of BM (p.o); and the fifth group received a sub protective dose of diazepam, i.e. 0.5 mg/kg and 50 mg/kg of BM. After an hour, all the animals were injected with 80 mg/kg pentylenetetrazol intraperitoneally and presence or absence of clonic convulsions was noted for each animal.

The % latencies of clonic convulsions were noted, and numerically transformed to a seizure score (S) calculated from the formula:

S = 1- (control Latency/ drug seizure Latency)

In the case of control animal; S = 0, whereas for animals that did not experience seizures latencies of infinity, S =1. This numerical transformation enabled inclusion of all animals in the statistical analysis, irrespective of whether they had a seizure or not. The mortality in the 24h following PTZ, in the different pretreatment groups was also recorded.

Statistical Analysis

The results are presented as mean ± SEM and subjected to "One-way ANOVA" followed by Dunnett's test of six animals in each group. The values of $p<0.05$ were considered significance.

RESULTS

PHYTOCHEMICAL SCREENING

The results of preliminary phytochemical screening of aqueous and chloroform exracts of *Enicostema axillare* non (Blume) A. Raynal., reveals the presence of Alkaloids ,Steroids, Triterpenoids, Flavonoids, Saponins, Tannins and Phenolic compounds etc.

Effects of AEEA & CEEA on MES Induced convulsion

The duration of tonic hindleg extension in rats treated with vehicle was 12.21±0.03seconds. The AEEA & CEEA at doses of 200 mg/kg and 400 mg/kg were protected the animals from seizures and significantly ($p<0.001$) reduced the duration of tonic hindleg extension for 4.92±0.1.61, 2.32±0.31 and 3.84±0.1.69, 2.24±0.54 seconds respectively. Whereas, the standard drug phenytoin treated animals exhibits abolished tonic hindleg extension. Phenytoin treated animals have shown 100% protection against MES induced seizures where as AEEA & CEEA 200 mg/kg and 400 mg/kg have shown 62.75%, 82.43% and 70.93%, 83.04% protection respectively.

Effect of AEEA & CEEA on PTZ Induced convulsion

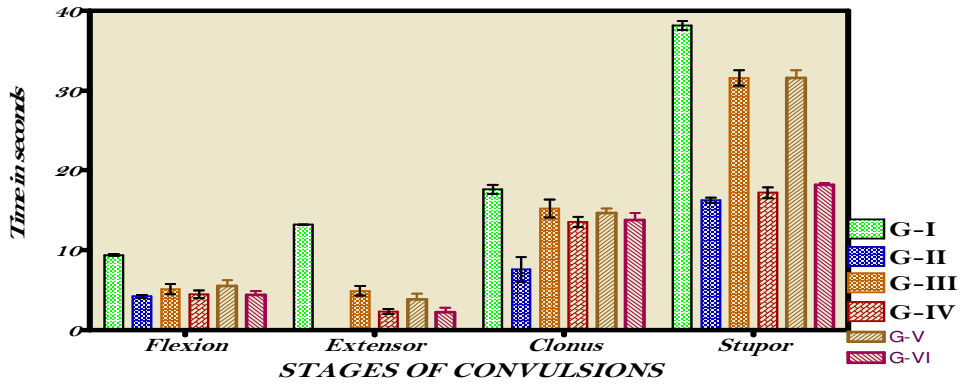
In rats treated with vehicle, clonic convulsion appeared for 187.75±0.668 seconds after PTZ and all rats died after seizures. The AEEA & CEEA at doses of 200 mg/kg and 400mg/kg significantly delayed the onset of clonic convulsions for 486.92±2.36, 594.64±2.37 ($p<0.001$) and 497.74±1.42, 497.74±1.42 ($p<0.001$) seconds respectively in dose dependent manner. Whereas, the standard drug diazepam (4mg/kg, i.p) delayed the onset of clonic convulsions for 695.31±2.32($p<0.001$) seconds. Diazepam treated animals have shown 100% protection against PTZ induced seizures where as AEEA & CEEA 200 mg/kg and 400 mg/kg have shown 70.02%, 85.52% and 71.58%, 82.67% protection of convulsion and 83.33%, 100% and 83.33%, 100% protection of mortality respectively.

Effect of *Enicostema axillare* extracts on MES induced seizures in rats

Group	Design of treatment	Flexion	Extensor	Clonus	Stupor	Recovery	% protection
I	Vehicle control	9.4±0.12	13.21±0.03	17.64±0.54	38.2±0.58	192.24	0
II	Phenytoin 25mg/kg.i.p.	4.2±0.23**	0**	7.59±1.54**	16.29±0.35*	93.5	100
III	AEEA 200mg/kg.p.o	5.12±0.62*	4.92±0.61**	15.24±1.14*	31.58±0.95	136.70	62.75
IV	AEEA 400mg/kg.p.o	4.49±0.49**	2.32±0.31**	13.52±0.65**	17.23±0.69*	138.54	82.43
V	CEEA 200mg/kg.p.o	5.52±0.72*	3.84±0.69**	14.64±0.56*	31.58±0.95	156.79	70.93
VI	CEEA 400mg/kg.p.o	4.42±0.47**	2.24±0.54**	13.82±0.85**	18.23±0.19*	134.51	83.04

Values are expressed as mean±SEM of six observations.
*p<0.05; **p<0.01. Comparison between Group I *Vs* Group II, Group III & Group IV
Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test.

Effect of *Enicostema axillare Extracts* on MES induced seizures in rats



DISCUSSION AND CONCLUSION

The whole plant of *Enicostema axillare* was collected from Tirupati, Chittoor (Dist.), Andhra Pradesh, India in the month of January, 2011. The taxonomical identification of the plant was done by Dr. K. Madhava chetty, Assistant Professor, Department of Botany, Sri Venkateshwara University (SVU), Tirupathi.

Review of literature explains mainly ethno botanical, ethno medicinal uses and pharmacological review. In this study the plant is used in epilepsy, inflammation, cancer, diabetis, abdominal ulcers, hernia, and hepatic disorders.

The powdered whole plant of *Enicostema axillare* was individually extracted with chloroform and water by continuous hot percolation method and maceration respectively. The colour of the extracts and percentage of extracts (11.6% w/w) were noted.

The preliminary phytochemical screening of the extracts *Enicostema axillare* showed the presence of alkaloids, flavonoids, tannins, saponins and Steroids. Preliminary organic analysis of drugs helps to undertake further studies on the isolation and identification of specific chemical constituents.

Acute toxicity study was done using OECD-423 (acute toxic class method). The extract did not show any mortality or signs of toxicity in experimental animals (rats).

The whole plant extract of *Enicostema axillare* were subjected to anti epileptic activity on male albino wistar rats. It was found that treatment with AEEA & CEEA on PTZ induced rats significantly reduce the duration of convulsion and delayed the onset of clonic convulsion. Although animal models based on Pentylenetetrazole (e.g. pentylenetetrazole threshold, and acute convulsions) have still been widely used for drug

screening, the mechanism by which pentylenetetrazole elicits its action has not been completely understood. One pentylenetetrazole exerts its action is by acting as an antagonist at the picrotoxin sensitive site of the $GABA_A$ receptor complex (Ramanjaneyulu and Ticku, 1984). Thus the present work substantiates the use of *Enicostema axillare* as potent anti-epileptic drug.

In this work, Phytochemical and Pharmacological activity of *Enicostema axillare* were studied. These parameters help in standardization of these generally accepted mechanism by which drugs but also aid in formulating Pharmacopoeial standards of drugs.

The results presented in this report are a preliminary communication. Further studies using the extract of *Enicostema axillare* are indicated to identify optimal treatment routes, dosage and which constituents may be conferring its anti-epileptic potential on this natural product.

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