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Research Article

Anti-Epileptic Activity of *Acalypha indica* Methanolic Leaves Extract with Animal Experiment

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ABSTRACT

Epilepsy is a set of chronic neurological disorders characterized by seizures. Nearly 90% of epileptic patients are found in developing countries. Epileptic seizures result from abnormal, excessive or hyper synchronous neuronal activity in the brain. The cause of most cases of epilepsy is unknown, although some people develop epilepsy as the result of brain injury, stroke, brain tumor, drug and alcohol exploitation. *Acalypha indica* is one of the species of the Euphobiaceae family. *Acalypha indica* shows anticonvulsant activity apart from many other medicinal properties such as anti-microbial, anti-inflammatory, and anti-oxidant. Fecl3 induced epilepsy in sprague dawly rats were used to study anticonvulsant activity of methonolic extract of *Acalypha indica*. The methanolic extract of *Acalypha indica* leaves was administered orally in graded doses of 200 mg/kg sprague dawly rats and the effects were compared with diazepam as standard and normal saline as control in $fecl_3$ induced method. The *Acalypha indica* leaf extract has shown significant decrease in the duration of tonic hind limb extension suggesting anticonvulsant effect. The performance results indicate that methanolic extract of *Acalypha indica* leaf extract have potential anticonvulsant activity.

Keywords: epilepsy, $Fecl_3$ induced rats, Diazepam, Sprague dawly, CNS, WDS *Acalypha indica*.

INTRODUCTION

Epilepsy is characterized by seizures one of the major neurological disorders. Some definitions of epilepsy report that seizures to be recurrent and unprovoked (Chang BS et al. 2003)[1] (WHO.2009; AAN.2012) but others report only a single seizure combined with brain alterations which increase the chance of future seizures. Modern drug therapy for epilepsy is complicated which includes side-effects, teratogenic effects, long-term toxicity. About 40% patients are refractory to therapeutic intervention and thus its effective and safe therapy remains a dispute. Epileptic seizures result from abnormal, excessive or hyper synchronous neuronal activity in the brain (Fisher R et al., 2005)[2]. About 50 million people worldwide have epilepsy, and nearly 90% of epilepsy is reported in developing countries (WHO.2009). Epilepsy becomes more common as people age (Brodie, MJ 2009; Holmes .2008)[3]. Onset of new cases occur most often in infants and the elderly (Wyllie's treatment of epilepsy.2010. Epilepsy is usually controlled, but not cured, with medication. However, over 30% of people with epilepsy do not have seizure control even with the best available medications. Surgery may be considered in difficult cases (Cascino GD. 1994; Engel J Jr.1996)[4]. 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 differing symptoms, all involving episodic abnormal electrical activity in the brain and numerous seizures. All the currently available antiepileptic drugs are synthetic molecules. Medicinal plants used for the therapy of epilepsy in traditional medicine have been shown to possess potential anticonvulsant activities in animal models anticonvulsant screening of animals for epilepsy can be valuable source for search of new antiepileptic compounds. Plants have been a principal source of traditional medicine for more than 5000 years. Plants and their phytoconstituents have important role in the development of a potent anti-convulsant agent. The use of plants and plant preparations has been in existence since pre- historic times. There are several reports on the use of plants in traditional healing. The available synthetic antibiotics are found to have serious side effects like bone marrow depression, anaemia and damage to vital organs like kidney. So it is necessary to identify newer antibiotics from herbal sources which are devoid of such serious side-effects. Therefore, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against drug resistant microbe strains. Medicinal plants are rich source of antimicrobial agents. Antimicrobial activities of many plants have been reported by the researchers. Antioxidants prevent the oxidative damage by directly reacting with ROS, (Since ancient times, many herbs have been potentially used as an alternative remedy for treatment of many infections, diseases and as food preservatives suggesting the presence of antimicrobial and antioxidant constituents. Antibacterial and antifungal activities of *Acalypha indica leaves* have been previously reported Chemical constituents of *Acalypha indica* L have been investigated. Anti-epileptic activity of *Acalypha indica* L

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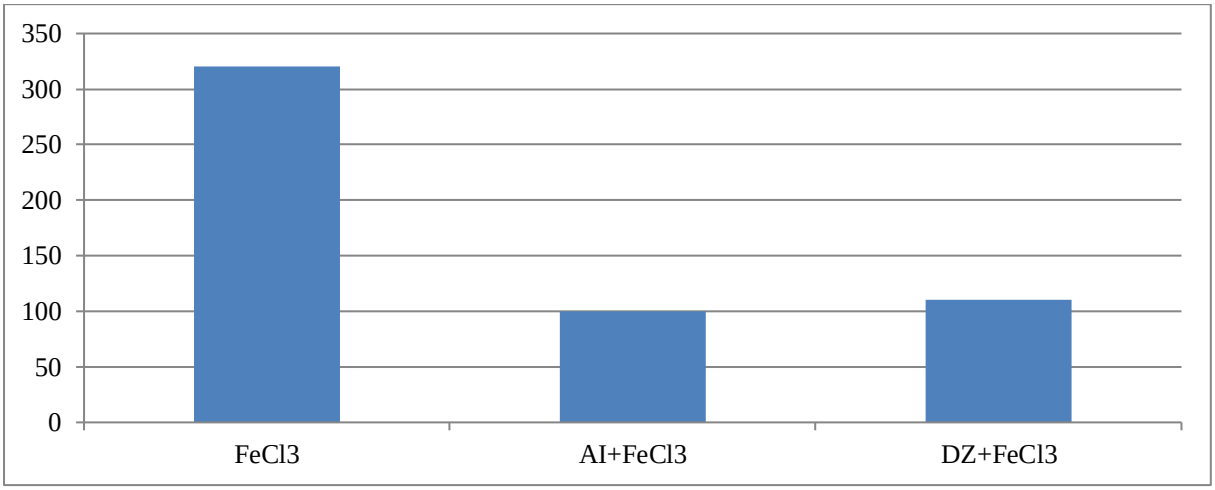

Figure 1: Effect of pretreatment of a methanol leaf extract of *Acalypha indica* (AI) and diazepam (DZ) on FeCl3 induced Wet Dog Scores (WDS). AI (200 mg/kg) and DZ (20 mg/kg) reduced WDS counts. Values are e expressed as Mean± SEM (n=6). * P< 0.05; ** P<0.01.

have not been investigated yet. Several medicinal plants are used to treat epilepsy and convulsions.[1]In view of the limited data on these plants it was the aim of this study to attempt to provide further information on the phenolic content and antioxidant activities of this plant using antioxidant assays.

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to humans against infections and degenerative diseases; however, recent concern has been paramount regarding the potential detrimental side effects of synthetic additives in humans. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemicals or microbiologic agents. There are many causes for the inflammations, but the mechanisms are common to all. There are two basic types of inflammation – acute and chronic. Acute inflammation is of short duration, which could be anything from a few minutes to a few days. Such inflammation is caused by foreign substances entering the body, or by physical damage. Viral infection may also precipitate acute inflammation. Chronic inflammation, on the other hand, is long lasting. It may persist for weeks, months or even years. Chronic inflammation may be brought on by acute inflammation or it may be the result of an auto immune disease. There is a need for a potent anti-epileptic agent, which is devoid of side-effects. Glutamate is a major excitatory neurotransmitter in the CNS. Exposure of neurons to high concentration of glutamate can lead to neuronal damage Currently, many drugs are available for treating this disorder, but these drugs have drawn backs like teratogenicity and other dose-related side effects. In spite of daily treatment, nearly 30% of patients continue to have convulsions and fail to provide a complete cure Wide range of medicinal plants have been identified by the ancient systems of medicines for treating these problems which are devoid of unwanted effects and are gaining popularity in most of the developing countries.

Importance of Acalypha indica in Ayurveda

Habitat: it is a Common annual shrub in Indian gardens, backyards of houses and waste place

Throughout the plains of India.

Plant Parts Used: Leaves

Constituents: Alkaloids “acalypus” and “acalyphine.”

Action: Cathartic, Anthelmintic, expectorant, emetic, anodyne and hypnotic

Preparations - Infusion of root, powder, decoction, tincture and liquid extract.

Uses:

Leaves possess laxative properties; “are used as a substitute for senega”; are used in the form of powder or decoction. Mixed with garlic they are used as Anthelmintic in worms. Mixed with garlic they are applied to scabies; and their juice mixed with oil forms an application in rheumatic arthritis. The pharmacological evaluation of substances from plants is an established method for the identification of lead compounds which can leads to the development of novel and safe medicinal agents. Expressed juice of the leaves is a safe, certain and speedy emetic for children in one teaspoonful (I drachm) doses, in cases of croup; in smaller doses it is expectorant, and is useful in chronic bronchitis, asthma. The decoction is employed in ear-ache as instillation and also as fomentation around the aching ear; and a cataplasm of the bruised leaves is applied to syphilitic ulcers, to maggot-eaten sores and also to relieve the pain of snakebites. Juice from fresh leaves may be employed in scabies and other skin diseases, and with lime and onion it is a good stimulating application for rheumatism. Powder of dry leaves is used in bed sores. In congestive headache a piece of cotton saturated with the expressed juice of the plant or leaves and inserted into each nostril is said to relieve it by causing haemorrhage from the nose. In cases of obstinate constipation of children, the leaves are ground into a paste and made into a ball and introduced into the rectum, an infusion of the root or the root bruised in water, acts as a cathartic.

MATERIALS AND METHODS

Mature leaves of *Acalypha indica* were collected from local fields of Hyderabad and Authenticated by Dr.

Table 1: Seizures Observation

Group I	Normal Healthy Rats	Normal diet and food	No seizures	No seizures	No seizures	No seizures
Group 2	Saline Treated Control Rats	Saline induction of intra peritoneal	No seizures	No seizures	No seizures	No seizures
Group 3	$Fecl_3$ Induced Epileptic Rats	Fecl3 intraperitonial injection	seizures observed	Head nodding, tail jerking	WDS was observed, facial movement	Seizures. Behavioral change
Group 4	Induction of Plant material	Oral induction of plant material	seizures observed	Head nodding, tail jerking	WDS was never observed, facial movement	Reduction of seizures
Group 5	Induction of diazepam	Diazepam induction	seizures observed	Head nodding, tail jerking	WDS was never observed, facial movement	Reduction of seizures

Shashikanth, Taxonomist, Department of Botany, Osmania University, Hyderabad-7. Male Sprague dawly rats of either weighing 200 to 250 gm were obtained from NIN, Hyderabad

METHODS

Extraction

The leaves collected were washed thoroughly under running tap water for making dust free and were shade dried within a temperature range of 38^0C-40^0C.After drying, the leaves were ground to fine powder. For extraction of phytochemical compounds by Soxhlet's extractor various solvents viz. Petroleum ether, Chloroform, Ethyl acetate, Ethanol and methanol were used.

Preparation of leaf extracts Collection of plant & preparation of extract

Acalypha indica leaves were shade dried for 10 days and pulverized to coarse powder using a manual blender. Extraction with Petroleum ether, Chloroform, Ethyl acetate, Ethanol, methanol extract was done in Soxhlet's extractor. The *Acalypha indica* powder was extracted with methanol by continuous extraction in a soxhlet. After extraction the solvent was removed, typically by means of evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains was discarded. The extract was dried under vacuum, stored at room temperature and protected from direct sunlight.

Drugs

diazepam, and 0.9%, normal saline (0.9% NaCl solution); $fecl_3$

were used in this study.

Experimental animals

Male Sprague dawly rats of either weighing 200 to 250 gm were obtained from NIN, Hyderabad. The animals were housed in standard cages with free access to food (standard laboratory pellet diet) and water. The animal house temperature was maintained at 23 ± 5.00C with a 12-h light/dark cycle. Experimental protocol was approved by Institutional Animal Ethical Committee (IAEC). The guidelines for the investigation of experimental seizures in conscious animals were followed. Based on the results obtained from this study, the dose of methanolic extract of *Acalypha indica leaf* for anti-convulsant activity was fixed to be 200mg/kg body weight.

Experimental design

Rats were at random assigned into five groups, (n=5 per group).

Group-I: Normal

Group II – Saline Treated Control Rats.

Group III – $Fecl_3$ Induced Epileptic Rats.

Group IV –$Fecl_3$ + Induction of Plant material.

Group V - $Fecl_3$ + Induction of diazepam after adaptation, Saline 25 ml/kg, (control), Group-II: diazepam 25mg/kg, (standard), Group-III, IV, V: Received *acalypha indica* plant extract doses. Five rats in the control group were injected with saline into intraperetonial injection. The five rats of epilepsy model group were injected with 20µl Ferric Chloride ($FeCl_3$) intraperetonial injection. And the five rats in plant materials induction treatment group were injected with 20µl Ferric Chloride ($FeCl_3$) (as above). Plant material is fed to rats after 1hr $FeCl_3$ injection. Behavioral manifestations in $FeCl_3$ induced epilepsy. Administration of $FeCl_3$ resulted in development of WDS in three groups of animals ($FeCl_3$, $FeCl_3$+M AI, $FeCl_3$ +DZ) whereas WDS was never observed after intra peritoneal injection of saline in control group of animals. Significant reduction in WDS counts was observed in AI pretreated $FeCl_3$ injected group ($P<0.01$) and in DZ pretreated $FeCl_3$ injected group ($P<0.01$) in comparison too untreated $FeCl_3$ injected group of animals. None of the animals have shown behavioral evidences of seizures

Induction of epilepsy by ferrichloride intra peritoneal injection:

A single injection of 10µl of ferric chloride into Rat (intra peritoneal) resulted in chronic recurrent focal peroxysomal discharges as well as behavioural conversions seizures. Iron filled macrophages, ferruginated reasons, and astrological cells surrounded the forms of like seizure discharge. Recurrent focal epileptic farm discharge caused by intra peritoneal rejection of iron salts suggested that the development of human epilepsy may depend, in part, a neuro chemical alterations induced by the principal

Table 2: Behavioural observations

	No seizures	Grade-I	Grade-II	Grade-III	Grade-IV
Observations	free from transient discharges	Isolated	Frequent isolated spikes along with cumulative spikes	Frequent burst of spikes	Long episode of epileptic spikes
Behavioral observations	None observed	None observed	Steadfast posture pauses the behavior	Head nodding movements facial movements	Shivering head nodding movements
Duration of transient epileptogenic episodes	Nill	>1s to<5s	1-5s	1-10s	5-20s
Frequency composition amplitude	Nill	3 (or)4 Hz α 250μv	6-7Hz α 250μv	α 250μv	7-10Hz α 250μv

metallic ions found in whole blood. The procedure for the location of epileptogenic foci and the diagnosis of drug resistance epilepsy has been described previously in our recent study. Samples were taken following anterior temporal cobectomy for rat's seizure free.

Behavioural Test

Open field behaviour tests: Behavioural Tests

Open field tests were performed in all rats 1 week after induction of epilepsy in a 106cm x 70cm x 70cm unroofed rectangular wooden box. (John P.J. Pinel, Dallas Treit)[5] Its exploratory behaviour was recorded for 15min and quantified. The distance moved horizontally in cms was quantified as the number of frames if stood on its hind limbs (rearing). The number of faecal boli expelled during the 15 min period was counted as the defecation index. Before each test the field was cleared thoroughly with 0.1% acetic acid solution.

Morris Water Maze Test

Morris water maze tests were conducted 8 days after induction of epilepsy in all rats. (Okaichi Y Amano S, Ihara N,)[6] In circulating tank (1.68m in diameter and 0.5m in depth) that was filled with water and divided into four quadrants with extra maze cause of different shapes size and colours in the above water part.

Water temperature was controlled at 22 to 25ºC.A black circular platform with a 15cm diameter was positioned 2.0cm under the surface of the water at the centre of one quadrant so that the rat could stand and escape by swimming to the platform. After surgery and 1day before the test each rat was placed once in the water for 129 ºC to adopt into the environment. During the test each rat was placed inside the water tank facing the tank wall at one of four randomly selected entry points, and its swimming ability was evaluated by the latency of the rat to reach the visible platform. If the animal failed to reach the platform with in 120s.It was guided to the platform and allowed to remain ---on the platform for 20s.Each rat was tested once a day for 4 consecutive days, starting from the day after surgery. On day 5 the platform was removed and the length of time that each rat spent in the assigned platform quadrant was recorded. The Morris water maze test was videoed by web camera mounted on the ceiling above the tank. The test was performed from 11.00h to 14.00 h to exclude variations resulting from the circadicum rhythm.

RESULTS

Epilepsy and Analysis: Behavioral observations were noted for 4 h after administration of FeCl3 solution. Animals were placed in individual observation chambers. The frequencies of WDS were recorded throughout a 1 hr period by observers with no knowledge of the treatment given to the rats. WDS was identified as a rapid rhythmic shaking of the head in a radial motion. The signs were counted and presented as number of events per hour**.** No seizures were observed in the Normal healthy group, but spontaneous seizures were observed in all three groups were graded according to the criteria. Only grade I or II were observed in the $Fecl_3$ reduced epileptic rats. Whereas Grade III & IV seizures were observed in the $Fecl_3$ induced epileptic rats were treated with *acalypha indica* plant extract and standard. The mean +/- SE number of seizures observed in a 5h period in the treated $Fecl_3$ induced epileptic rats. (12.13+/-1.25) was higher than in the saline treated control group (0.37+/-8.25: P α 0.05) but these were significantly lower than the Mean Number of seizure observed in the $Fecl_3$ induced epileptic rat.

Acalypha indica methanolic Plant material considerably decreased the number and Grade of Seizures and enhanced Rat behaviour, compared with untreated epileptic Rats. Crude extract of Plant reduced the levels of BDNF protein in the fore brain and increased levels of BDNF protein in the hippocampus compared with untreated epileptic rats. *Acalypha indica* methanolic extract inhibited seizures in $fecl_3$ induced epileptic Rats and reported their behaviour. Criteria used for different grades of seizures manifested by untreated $Fecl_3$ - Induced epileptic and Acalypha indica methanolic extract treated. $Fecl_3$ -Induced Epileptic Rats.

Behaviour Tests

Open Field Behaviour Tests: Ambulation, Exploration and defecation were monitored in an open field Test. Epileptic Rats exhibited significantly decreased ambulatory activity. compared with rats in the normal and saline treated control groups ($P<0.05$ for all comparisons). *Acalypha indica* methanolic extract treatment of $Fecl_3$ induced epileptic rats significantly reduced the defecation index, increased the ambulatory activity compared with rats in the $Fecl_3$ induced epileptic group.

Morris water maze Test

In the morris water maze test at day 4, *Acalypha indica* methanolic treated $Fecl_3$ induced epileptic rats exhibited

similar latency to rats in the normal healthy group and saline treated control groups.

Rats in all three of these groups had significantly shorter latency in finding the platform compared with $Fecl_3$ induced rats in the quadrant where the platform had previously been placed was significantly longer (mean +/-SE,33.47+/-5.20s, $p<0.05$) compared with $Fecl_3$ induced epileptic rats (mean +/-SE,21.57+/- 4.83s) and slightly longer than in the saline treated controlled group. Behavioural observations; Epilepsy is a CNS disorder associated with recurrent episodes of seizures due to the abnormal electrical activity in The brain. In India, 5.59 per 1000 population are affected by this disorder. The currentTreating epilepsy is associated with many adverse effects. Hence, there is a need for a potent anti-convulsant agent. Which is devoid of side-effects. Plants and their phytoconstituents have important role in the development.

DISCUSSION

Acalypha indica is one of the widely used species of the eupharbiacea and is a common condiment for various foods and beverages. It is used for a wide array of unrelated ailments that Include arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, Vomiting, hypertension, dementia, fever, infectious diseases and helminthiasis. *Acalyph indica* and its active compounds.(Okaichi Y[1], Amano S, Ihara N)[7] Found to have immuno-modulatory, anti-tumorogenic, anti-inflammatory, anti-apoptotic, anti-hyperglycaemic, antilipidemic. Leaves are used in the form of powder or decoction; Mixed with garlic they are applied to scabies; and their juice mixed with oil forms an application in rheumatic arthritis. Expressed juice of the leaves is a safe, certain and speedy emetic for children in one teaspoonful (I drachm) doses, in cases of croup; in smaller doses it is expectorant, and is useful in chronic bronchitis, asthma and consumption. Decoction is employed in ear-ache as instillation and also as fomentation around the aching ear; and a cataplasm of the bruised leaves is applied to syphilitic ulcers, to maggoteaten sores and also to relieve the pain of snakebites (Santra*b* Dr. J. Raamachandran)[8]. from fresh leaves may be employed in scabies and other skin diseases, and with lime and onion it is a good stimulating application in rheumatism. Powder of dry leaves is used in bed sores. In congestive headache a piece of cotton saturated with the expressed juice of the plant or leaves and inserted into each nostril is said to relieve it by causing hemorrhage from the nose (Schmelzer, G.H. & Gurib-Fakim, A.)[9]. In cases of obstinate constipation of children, the leaves ground into a paste and made into a ball and introduced into the rectum, relax the sphincter ani and produces free motions. And anti-emetic actions. Acalypha is a strong anti-oxidant agent and may prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse effects. Fecl3-induced convulsion model is a widely used tool to screen drugs for generalized tonic-clonic seizures. Causes several changes at the cellular level, disrupting the signal transduction in the neurons. Fecl3 inducing causes cellular Damage by facilitating the entry of Ca 2+ into the cells in large amounts, prolonging the duration of Convulsions. Apart from Ca 2+ ions, MES may also facilitate the entry of other positive ions like Na +, blockade of which, can prevent the MES-induced tonic extension. Currently available anticonvulsant drugs like sodium. Valproate and phenytoin act by modulation of these ion channels. On the other hand, drugs that antagonizes. NMDA receptors or potentiate opioids and GABA receptors are also reported to protect against fecl3-induced epielepsy. Seizures (methanolic extract of *acalypha indica* leaves) exhibited a significant ($P<0.01$) dose dependent, Protection against tonic extensor phase at all tested doses with maximal effect seen in Higher dose (200 mg/kg). This observed effect suggests that the protection of acalypha methanolic extract was maximum at 200 mg/kg. The anti-convulsant activity can be due to the presence of various phytoconstituents like *Acalyphine.* It has been suggested that fecl3 induced convulsions are associated with oxidative damage. *Acalypha* also has strong antioxidant property. The anti-convulsant activity of *Acalypha indica.*

CONCLUSION

The present study demonstrated that methanolic extract of *acalypha indica* leaves has dose dependent. Anticonvulsant activity in tested animal model. Further research is required to elucidate specific mechanism and. active principles responsible for its anticonvulsant property. Based on the above investigations, it may be concluded that the methanolic extract of leaves of *A. Indica* exhibited significant antiepileptic activity. These conclusions validate the traditional use of this plant in the control and/or treatment of convulsions and epilepsy as the plant is uncultivated yet. The presence of flavonoids may partially contribute the significant activity of methanolic extract of aerial parts of *Acalypha Indica* by improved gabaergic. *Acalypha indica* methanolic extract inhibited seizures in $fecl_3$ induced epileptic Rats and reported their behavior. These effects might be mediated by altering BDNF protein levels in the Brain. Criteria used for different grades of seizures manifested by untreated, $Fecl_3$ - Induced epileptic and *Acalypha indica* methanolic extract treated. $Fecl_3$ -Induced Epileptic Rats. Based on the results of the present study, it may be concluded that the methanol extract of AI (*Acalypha indica*) has potential antioxidant and antiepileptic action. The present study also encourages further investigations for existence of any principle compounds modulating the neurotransmitter receptor interaction and more importantly as the use of the plant product as medicine for reducing the chances of occurrence of epilepsy**.**

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