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Research Article

ANTICONVULSANT ACTIVITY OF *ANACARDIUM OCCIDENTALE* L. LEAVES EXTRACT IN EXPERIMENTAL MICE

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ABSTRACT

Objective: The present study was undertaken to investigate the anticonvulsant effects of *Anacardium occidentale* L. leaves in animals models.

Materials and Methods: Swiss albino mice were used for the study. Acute toxicity and neurotoxicity studies were performed. The extracts and standard drugs were administered once daily for a period of 14 days. Maximal electroshock induced convulsion and strychnine induced convulsion models were used for the study. Phenytoin and Diazepam were used as standard agents throughout the study.

Results and discussion: The extract did not show any type of toxicity.Anticonvulsant studies with EEAO showed a significant protection in MES induced convulsion models in a dose dependent manner. There was a significant ($P < 0.05$) decrease in the duration of tonic hind limb extension at both the doses of extract (200 and 400mg/kg) in MES model. Compared with the control group, treatment with EAAO had no significant effect on onset and duration of convulsions in the strychnine induced seizure model. As expected, the animals treated with diazepam 4 mg/kg increased onset , duration of convulsions and latency to death as compared with control group. In all groups, all animals had seizures and all died.The litreture reveals the presence of flavonoids,glycosides tannins etc.

Conclusion: The presence the chemical constituents gave strength to its anticonvulsant action. However, further research is warranted to determine the specific mode of its anticonvulsant activity.

Keywords: *Anacardium occidentale,* Strychnine, Seizure, Neurotoxicity.

INTRODUCTION

Epilepsy is defined as a chronic disorder of the brain that is characterized by spontaneous and recurrent seizure activity, which is triggered by the abnormal discharge of neurons [1]. Worldwide, this disease directly affects more than 50 million people and is roughly in the range of 5-10 per 1000 people and 100-190 per 100,000 people in industrialized and developing countries respectively [2]. Although several anticonvulsant drugs are used to treat seizure attacks, about 30% of patients are medicated incompletely. Furthermore, current antiepileptic drugs have toxicity and teratogenic effects [3]. This consideration implicates search for the new antiepileptic agents having lesser side-effects and quick on set of action.

Anacardium occidentale (AO) is a tree native to tropical America (Mexico, Peru, Brazil, etc.) and belongs to the family *Anacardiaceae*. Despite that, it is widely cultivated in India and East Africa; India being its largest producer [4]. In Folk medicine, in West Africa, as well as in South America, decoction or the leaves has been used to treat gastrointestinal disorders. The cardiovascular effects of the aqueous extract of the cashew tree leaves have been studied on the arterial blood pressure of the rabbit. The anti-microbial effect of 80% ethanol extract of the cashew tree leaves has been described by Kudi *et al.* [5]. Furthermore, cashew nut occupies a central position in the diets of the human population throughout the world, and it has been proved that its consumption has a cardio protective, anti-obesity, anti-cancer and antioxidant effects [6]. In fact, generally nuts including cashew nuts have been suggested as a natural source of antioxidants such as phenolics, flavonoids, tocopherols and alkyl-phenols [7]. The leaves possess antidiabetic [8], antiulcer [9] and anti-inflammatory [10] activities. The present study was undertaken to investigate the anticonvulsant effects of AO L. leaves in maximal electro shock and strychnine induced seizures model.

MATERIALS AND METHODS

Plant material

The leaves of cashew tree (AO) were collected from Goa during the 2011 season and authenticated by Prof. R.R. Singh, Head, Department of Botany, Lucknow University, Lucknow, Uttar Pradesh, India and the voucher herbarium specimen was deposited in the Department of Botany, Lucknow University, Lucknow, for future reference. The samples were washed, and air dried and this was followed by complete drying in an oven at 400°C. The dried sample was crushed mechanically to powder, sieved and stored in an air-tight container for further analysis.

Preparation of the extract

The powdered was extracted with different solvents of varying polarity by sox let apparatus at room temperature. The extracts were evaporated to dryness on a rotary evaporator at 37°C and the residues were kept for further analysis.

Animals

Swiss albino mice (weighing between 18 and 25 g) obtained from the animal house of Babu Banarasi Das National Institute of Technology and Management (BBDNITM), Lucknow were used in the study. They were maintained at a temperature of 22 ± 5°C, relative humidity 55 ± 5°C with free access to food and water ad libitum, under 12:12 light/dark cycle (light on at 8:00 hr). All manipulations were carried out only once between 9:00 and 15:00 hr. with each animal used.

The experimental protocol was approved by the Institutional Animal Ethics Committee as per the direction of the Committee for the Purpose of Control and Supervision of Experimental on Animals (approval no BBDGEI/IAEC/29/2011). The animals were acclimatized for a period of 7 days before the study. All efforts were made to reduce the number of animals used and treated humanely to minimize their pain and discomfort.

Drugs and chemicals
Phenytoin (Orgamine Chemicals Pvt. Ltd., Thane), Diazepam (ALPA Laboratories Limited, Pigdamber), Strychnine (Sigma Aldrich, USA) were used as standard drugs. All other chemicals and reagents used for the study were of analytical grade.

Acute toxicity study
Mice were kept on overnight fasting and water was withdrawn 3-4 before administration of test compound. Ethanolic extract of *A. occidentale* (EEAO) was administered orally in increasing doses of 100, 500, 1000, 2000 and 4000 mg/kg body weight. Immediately after dosing, the mice were observed continuously for 4 hrs for symptoms of toxicity like motor activity, tremors, convulsions, tonic extension, muscle spasm, loss of frighting reflex, ataxia, sedation, hypnosis, lacrimation, diarrhea, salivation and writhing. Mice were then kept under observation up to 72 hrs for any mortality [11]. Locomotor activity was monitored using actophotometer (IMCORP, India), animals were individually placed in activity meter after 60 minutes of treatment and total activity count was registered for 5 minutes. The locomotor activity was expressed in terms of total photobeam interruption counts/5 minutes [12].

Neurotoxicological studies
Neurotoxicity was determined using rotarod test. Mice, which were able to remain on the rotating rod, with a speed of 10 rpm for 5 minutes or more were selected and divided into three groups (n=6). The experimental groups received varying doses of extract 200, 400 mg/kg (p.o.). One group received only vehicle and served as control. All animals were placed on the rotarod after 60 minutes of the treatment and average retention time on the rod was calculated. Neurotoxicity was assessed as inability of the animal to maintain equilibrium on the rotating rod for at least 3 minutes [13,14].

Maximal electroshock (MES) induced convulsion
Animals were divided randomly into four groups of six mice in each group. Group 1 (Control group) received vehicle by oral route; Group 2 (standard group) received phenytoin (25 mg/kg) by intraperitoneal route. Group 3 and Group 4 were treated with EEAO 200 and 400 mg/kg, p.o. respectively once daily for 14 days. On 14th day 60 min after treatments, 50 mA current for 0.2 seconds was administered through corneal electrodes to induce convulsions. The ability of the drugs to prevent or delay the onset of hind limb extension was taken as an indication of anticonvulsant activity [15,16].

Strychnine induced seizure
The grouping and treatments of animals were same as MES model. On 14th day 60 minutes after treatments strychnine was injected intraperitoneally at the dose of 2.5 mg/kg. The onset and duration of seizure, onset of death and % protection were assessed for each animal [17].

Statistical analysis
The different results are expressed as mean ± standard error of the mean. The comparisons between the averages of the series of values were performed using ANOVA test, followed by post-Tukey test.

RESULTS

Acute toxicity
In acute toxicity study, EEAO did not show any mortality in mice. Even at this higher dose i.e., 4000 mg/kg, there were no gross behavioral changes were observed, and 200 mg/kg and 400 mg/kg dose were used for evaluation of anticonvulsant activity.

Neurotoxicological studies
In the rotarod test, the vehicle-treated mice did not demonstrate any signs of impaired motor co-ordination. Each control mouse was capable of performing the test, i.e., the mean time spent on the rotarod apparatus was 180 seconds. Similarly, EEAO did not impair motor coordination of mice in the rotarod test at any dose. Thus, the extract was found to have no neurotoxic effects up to 400 mg/kg dose (Table 1).

MES induced model
Anticonvulsant studies with EEAO showed a significant protection in MES induced convulsion models in a dose-dependent manner. There was a significant ($p<0.05$) decrease in the duration of tonic hindlimb extension at both the doses of the extract (200 and 400 mg/kg) in MES model with maximum protection observed at 400 mg/kg dose, when compared to control group. The anticonvulsant activity of the extract was found to be comparable to phenytoin treated group (Table 2).

Strychnine induced seizure
Compared with the control group, treatment with EAAO had no significant effect on onset and duration of convulsions in the strychnine induced seizure model. As expected, the animals treated with diazepam 4 mg/kg increased onset, duration of convulsions and latency to death when compared with the control group. In all groups, all animals had seizures and all died (Table 3).

DISCUSSION

The present study was designed to investigate the anticonvulsant activity of AO extract in MES and strychnine induced convulsions. The results of the study showed that EEAO possessed significant anticonvulsant effect against MES induced convulsions, but had a mild effect on strychnine induced convulsions.

There are a number of synthetic anticonvulsant drugs currently available for use in the management, control and/or treatment of individuals with epilepsy. The mechanisms of action of antiseizure (anticonvulsant) drugs have been broadly divided into three major categories [18]. According to McNamara [19], anticonvulsant drugs that are effective against the most common forms of epileptic seizures, namely partial and secondarily generalized tonicclonic seizures, appear to act either by (i) reducing or

Table 1: Acute toxicity and neurotoxicity screening of EEAO

Treatments	Acute toxicity test		Neurotoxicity screening
	Locomotor activity count/5 minutes	% mortality	Retention time (seconds)
Vehicle control	358±4.89	0	312.17±1.68
Extract 200 mg/kg	346.50±6.27	0	309.17±0.72
Extract 400 mg/kg	371±3.48	0	305.33±1.65

EEAO: Ethanolic extract of *Anacardium occidentale*

Table 2: Effect of EEAO on MES induce convulsion model in mice

Treatments	Extensor phase (seconds)	% protection
Vehicle control	20.83±0.41	0
Standard (phenytoin)	1.67±0.34***	100
Extract 200 mg/kg	19.17±0.32*	60
Extract 400 mg/kg	7.33±0.34***	80

All values are given in mean±SEM, *p<0.05, ***p<0.01 as compare with the control group (one-way ANOVA, followed by Tukey post-test). SEM: Standard error of the mean, MES: Maximal electroshock, EEAO: Ethanolic extract of *Anacardium occidentale*

Table 3: Effect of EEAO on strychnine induce seizure in mice

Treatments	Onset of seizure	Duration of seizure	Onset of death	% protection
Vehicle control	16.51±0.12	2.49±0.08	21.92±0.10	0
Standard (phenytoin)	22.82±0.10	1.02±0.07	27.64±0.13	60
Extract 200 mg/kg	18.69±0.07	1.50±0.02	22.54±0.10	0
Extract 400 mg/kg	19.96±0.07	1.01±0.06	24.00±0.12	40

All values are given in mean±SEM (one-way ANOVA, followed by Tukey post-test). SEM: Standard error of the mean, EEAO: Ethanolic extract of *Anacardium occidentale*

limiting the sustained repetitive firing of neurons, an effect mediated by promoting and/or prolonging the inactivated state of voltageactivated Na^+-channels, thereby reducing the ability of neurons to fire at high frequencies, or (ii) enhancing and facilitating gamma-aminobutyric acid (GABA)-mediated synaptic transmission and inhibition, an effect mediated either by a pre-or post-synaptic action. In the presence of GABA, the gamma-aminobutyric acid-A (GABAA) receptor is opened, thus allowing an influx of Cl- ions, which in turn increases membrane polarization. Some antiseizure drugs also act by reducing the metabolism of GABA [19]. Other anticonvulsants act at the GABAA receptors, enhancing Cl- ion influx in response to GABA, or by promoting GABA release [19]. Anticonvulsant drugs that are effective against absence seizure, a less common form of epileptic seizure, act by reducing or limiting the flow of Ca^{2+} through T-type voltage-activated Ca^{2+}-channels, thus reducing the pacemaker Ca^{2+} current that underlies the thalamic rhythm in spikes and waves seen in generalized absence seizures.

Almost all antiepileptic drugs show the signs of sedation, hypo or (less often) hyper-locomotion, ataxia, abnormal gait, reduced or inhibited righting reflexes and muscle relaxation in laboratory animals. These effects are commonly termed as neurotoxicity. In laboratory neurotoxicity can be determined using retard test. In a study used rotarod test to determine neurotoxic effects of AO extract. The extract showed no neurotoxicity as there was no sedation, normal gait, no change on righting reflexes, and all animals were able to maintain equilibrium on rotating the rod for more than 3 minutes.

In the central nervous system (CNS), the disruption of the naturally existing balance between the concentrations of inhibitory and excitatory neurotransmitters is thought to be the main cause of convulsive episodes. Electrical stimulation of certain areas of the brain results in a permanent lowering of after discharge threshold (AD) and the development of potentiality to trigger motor seizures in those areas. The lowering of AD threshold appears to be a local phenomenon, whereas the development of motor seizures involves changes that take place outside the stimulated structure [20,21]. Tissue damage or metallic ion deposits, for example, have been eliminated as a possible mechanism underlying the development of motor seizure by electrical stimulation [22]. Another possibility is that neuronal cells are being sensitized so that more and more cells near the electrode are being fired by stimulation as the treatment continues. The other hypothetical mechanism proposes an increase in strength of inter-limbic connections, so that a discharge in one structure more readily activates an independent discharge in several other structures with a consequent convergence into the "motor" structure. When enough secondary foci are triggered, the motor structure is driven which in turn drives the skeletal response [23].

The MES test is the most widely used animal model in the evaluation of antiepileptic drugs the MES test identifies agents with activity against generalized tonic clonic-seizures using clinically established antiepileptic drugs. MES causes several changes at the cellular level, which can disrupt the signal transduction in the neurons. One of the most important mechanism by which it causes cellular damage is facilitation of Ca^{2+} entry into the cells in large amounts and thus 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 convulsions [24]. MES induced seizures are abolished by the drugs that blocks voltage gated sodium channels like phenytoin and carbamazepine or by the drugs that block N-methyl-D-aspartate (NMDA) receptors like felbamate. Whereas the drugs that block T-type Ca^{2+} current in thalamus like sodium valproate [25]. Phenytoin, a widely utilized anticonvulsant drug, predominantly exhibits a significant anticonvulsant activity in MES test and is utilized in the control of convulsive-seizures in epileptic patients.

Chemoconvulsant models for primary generalized seizures include by bicuculine (GABAA receptor antagonized), strychnine (glycine receptor antagonist) and aminophylline (adenosinereceptor antagonist) [26]. These substances block the physiological inhibitory action of glycine by a non-competitive action. This effect might explain their epileptogenic nature. Strychnine-induced seizures are different from those produced by primary GABA antagonists since they are mainly extensor tonic. These seizures are not fully relieved by acceptable doses of any of the classical anticonvulsants including benzodiazepines [27].

Strychnine, competitive antagonist of glycine receptors in the spinal cord. Although glycine is thought to act as an inhibitory neurotransmitter, a strychnine-insensitive glytine (Gly,) receptor has been recently described in cultured mouse neurons that are thought to be allosterically linked to the excitatory amino acid NMDA receptor. The seizure potentiating effects of glycine are blocked by aminophosphonovaleric acid, an NMDA antagonist. In addition, in animals pretreated with a subconvulsive dose of strychnine to block strychnine-sensitive glycine receptors (Gly), glycine enhances, rather than inhibits, NMDA-induced convulsions. Together, these results indicate that the seizure-potentiating [28].

The observations emanated in anticonvulsant studies showed that the EEAO possesses anticonvulsant activity as evidenced by decrease duration of tonic hindlimb extension in MES induced convulsions and increased latency to clonic convulsions in strychnine induced convulsions in a dose-dependent manner. Extract was found to be more active against MES induced convulsions when compared to strychnine induced convulsions.

Several reports suggest that alkaloids, triterpenes steroids and flavonoids and fatty acids have potent antiepileptic effect in various seizure models. In addition to this, saponins have also been able to modulate the neurotransmitter levels in the brain and to possess potent anti-convulsant activity [29]. There are some evidences about anticonvulsant effects of fatty acids [30]. The fatty acid composition of neuronal membranes declines during aging, but dietary supplementation with essential fatty acids was shown to improve membrane fluidity and polyunsaturated fatty acids (PUFA) content. In addition to affecting membrane biophysical properties, PUFAs in the form of phospholipids in neuronal membranes can also directly participate in signaling cascades to promote neuronal function, synaptic plasticity and neuroprotection [31].

There has been a resurgence of interest in synthetic and plant-derived flavonoids as modulators of GABAA receptor function influencing inhibition mediated by the major inhibitory neurotransmitter GABA in the brain. Areas of interest include (i) flavonoids that show subtype selectivity in recombinant receptor studies *in vitro* consistent with their behavioral effects *in vivo*, (ii) flumazenil-insensitive modulation of GABAA receptor function by flavonoids, (iii) the ability of some flavonoids to act as second-order modulators of first-order modulation by benzodiazepines and (iv) the identification of the different sites of action of flavonoids on GABAA receptor complexes. An emerging area of interest is the activation of GABAA receptors by flavonoids in the absence of GABA [32]. The previous phytochemical studies confirmed the presence of alkaloids, triterpenes steroids, flavonoids and fatty acids in AO.

CONCLUSION

EEAO increased the threshold of MES and strychnine induced convulsions with no neurotoxic effects, in a dose-dependent manner. Pretreatment with the extract showed that the extract might be mediating its effect via modulating neurotransmitter level in CNS. The presence the chemical constituents gave strength to its anticonvulsant action. However, further research is warranted to determine the specific mode of its anticonvulsant activity.

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