INTERNATIONAL RESEARCH JOURNAL OF PHARMACY ISSN 2230 – 8407
Available online www.irjponline.com Research Article

AN EXPERIMENTAL EVALUATION OF ANTICONVULASANT ACTIVITY OF *NERIUM OLEANDER* LEAF EXTRACT

Rout Susanta Kumar*, Kar Durga Madhab
Department of Pharmacology,School Of Pharmaceutical Sciences, SOA University, Kalinga Nagar, Bhubaneswar, Odisha, India

Article Received on: 09/08/11 Revised on: 20/09/11 Approved for publication: 11/10/11

*Email: susanta_rout@rediffmail.com

ABSTRACT
Epilepsy is the most frequent neurodegenerative disease. It is a state of recurrent, spontaneous seizures. The present study was undertaken to evaluate the anti-convulsant activity of different extracts of *Nerium oleander*. Different doses of different extracts were assessed by using Maximal electro shock (MES) and Pentylene tetrazole (PTZ) induced convulsions for its anticonvulsant activity in a dose dependent manner. Anticonvulsant effect of the extracts were comparable to clinically used antiepileptic drugs Diazepam and phenytoin. The ethanolic and aqueous extracts showed significant activity in MES induced seizures by reducing tonic phase than compare to other extracts and control.The ethanolic extracts significantly delayed the onset of clonic and tonic convulsions induced by PTZ.
KEYWORDS: Anticonvulsant, *Nerium oleander*, Maximal Electroshock seizure (MES), Pentylenetetrazole(PTZ),

INTRODUCTION

Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is a disorder of brain characterize by unpredictable and periodic occurrence of a transient alteration of behaviour due to the disordered, synchronous and rhythmic firing of populations of brain neurons [1]. Epilepsy is one of the most common afflictions of human beings with a prevalence rate of approximately 1 % of the total population [2]. Incidence of epilepsy in developed countries is approximately 50 per 100,000 while that of developing country is 100 per 100,000 [3]. It has been observed that the presently available antiepileptic drugs are unable to control seizures effectively in as many as 25% of the patients [4]. The conventional antiepileptic agents like phenytoin, carbamazeipine and sodium valporate carry with them several serious side effects notably neurotoxicity [5]. As majority of antiepileptic drugs are consumed life long, concomitant administration of other drugs predisposes to the risk of drug interaction. However, newer antiepileptics like gabapentin, vigabatrin, lamotrigine, etc are used supplemental to the conventional agents [1]. Thus, it is necessary to investigate for an antiepileptic agent that is highly efficacious as well as safe in items of drug related toxicity. The aim of treating an epileptic is not only to abolish the occurrence of seizures but also to lead a self sustained life. The herbal medicines have wide therapeutic actions and safety profile. This makes the herbal therapies to be successful. One of these can be the use of *Nerium oleander* (Family: Apocyanaceae) also known as kanner is an important shurb used against various disorders in indigenous system of medicine. The shrub commonly grows. It is in leaf all year, in flower from June to October. The flowers are hermaphrodite [6]. Leaves are powerful repellent. A decoction of the leaves has been applied externally in the treatment of scabies and to reduce swellings. The leaves and the flowers are cardiotonic, diaphoretic, diuretic, emetic, expectorant. It has also being reported to have antibacterial and anti diabetic activities [7,8]. The *Nerium oleander* is an evergreen shrub growing to 4m by 4m. It is in leaf all year, in flower from June to October. The flowers are hermaphrodite [6]. The major components of it are oleander, neriin and oleandrin. The bark contains toxic glucosides, rosaginin and nerrin, volatile oil, fixed oil, etc [9]. In ancient ayurvedic literature, the plant is reported, to cure conditions of various skin disorders, wound ,vomiting, burning sensation, wandering of the mind, cough . The objective of present study investigated the anticonvulsant activity of the Pet.ether,ethanol and aqueous extracts using Maximal electroshock induced convulsion(MES) model and Pentylenetetrazole(PTZ) induced seizure model.

MATERIALS AND METHODS

Plant material: *Nerium oleander* leaves were collected from the rural area of Anandapur,Odisha. The plant was authenticated in the department of Bioscience, S.P.University , Anand, Gujarat, India. A voucher specimen of plant material was kept at School of Pharmaceutical sciences, SOA University, Bhubaneswar, India for future reference.

Preparation of the extract: The leaves were shade dried at room temperature and finely powdered with help of a hand-grinding mill in such a way that the powdered material passed through sieve no 40. The powered of leave of *Nerium oleander* was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents successively in increasing order of polarity from petroleum ether, alcohol and finally fresh aqueous extract [10]. The extracts were subjected to preliminary qualitatative chemical analysis [11]. The suspensions of all the extracts were prepared by using 0.5% Tween 80 in normal saline for the experiment.

Drugs and chemicals: Phenytoin sodium (Sun Pharmaceuticals India.Ltd, Halol. Gujarat. India,), Diazepam (Ranbaxy Laboratories Ltd, HMTD textiles, India), Pentylene tetrazole (Sigma-Aldrich, St.Louis, MO63103, USA) were used for the study. All the solvents used for the extraction process are of Laboratory grade and they are purchased from local firms. Fresh drug solutions were prepared on the day of the experiment and administered orally (P.O.).

Animals: Studies were carried out on *Wistar* albino rats of either sex weighing 150-220 g and Albino mice weighing between 18-22 g were used were obtained from animal house, School of Pharmaceutical Sciences, SOA University, Bhubaneswar, Odisha, India. Animals were housed in groups of six in standard laboratory conditions of temperature (25±2°C), relative humidity (55 %), lighting (08:00–20:00 h) with food and water freely available. They were fasted 8 h before the experiments, but allowed free access to water. The study was approved by the Institutional Animal Ethical Committee.

Acute toxicity studies (LD50): The acute oral toxicity study was carried out as per guidelines set by Organisation for Economic Cooperation and Development (OECD). The median lethal dose of the pet-ether alcohol and aqueous was determined by orally administering the extracts in increasing dose levels of 0.1,0.2,0.5, 1, and 2 g/kg body weight to healthy adult albino mices of either sex. The animals will be observed continuously for 2 h under the following profiles:

I. Behavioural profile: Alertness, restlessness, irritability and fearfulness.
II. Neurological profile: Spontaneous activity, reactivity, touches response, pain response and gait.
III. Autonomic profile: Defecation and urination.
After a period of 24 h they will be observed for any lethality or death (% of mortality).

Assessment of anticonvulsant activity

Maximal electroshock seizure (MES) test

The animals were divided into eleven groups. Group 1 received 1 ml/rat of saline, group 2 received 25 mg/kg of Phenytoin, groups 3, 4 and 5 received 75,150 and 300 mg/kg of petroleum ether, groups 6, 7, 8, 9, 10 and 11 received alcohol and aqueous extracts respectively of *Nerium oleander*. The saline and standard reference drug were administered 45 min before induction of seizure, where as the test extracts of *Nerium oleander* were administered 1 h before induction of seizure. To induce convulsions in the control and drug treated animals, the maximal (tonic hind limb extension) electroshock seizure (MES) was carried out via copper electrodes (introduced bilaterally into the ears) with the apparatus Electro-convulsometer (Techno), using a fixed current 150 mA in rats for 0.2 s. The tonic extension of the hind limbs (extensor phase) and mortality were recorded [12, 13].

Pentylenetetrazole (PTZ) Induced Convulsions:

In Pentylene tetrazole (PTZ) induced convulsion model ,the animals were divided into 8 groups of 6 animals each. Group I served as control. Group II served as standard group, and was treated with Diazepam (4 mg/kg) and pentylenetetrazol (70 mg/kg) i.p. Group III and IV were treated with alcoholic extract (150and 300mg/kg body weight respectively) orally and pentylenetetrazol (70 mg/kg) i.p. Group V and VI were treated with aqueous extract (150 and 300mg/kg body weight) orally and pentylenetetrazol (70 mg/kg) i.p. Group VII and VIII were treated with pet.ether extract (150 and 300mg/kg body weight) orally and pentylenetetrazol (70 mg/kg) i.p. The standard and control groups received diazepam (4mg/kg, ip) and 1% tween 80 (10 ml/kg, orally) respectively. PTZ was injected intraperitoneally to the extract treated and control groups after one hour and diazepam treated group after 30 mins. The animals were then individually placed in trays and observed immediately. After PTZ injection for a period of 30 minutes. The latency and duration of myoclonic jerks as well as incidence of seizures were recorded. Time taken for death/recovery was also noted [14].

RESULTS AND DISCUSSION

Epilepsy is one of the most common serious neurological conditions. Seizure refers to a transient alteration of behaviour due to disordered, synchronous and rhythmic firing of populations of brain neurons [15]; the frequency and importance of epilepsy can hardly be overstated from the epidemiologic studies. However, in most studies, the overall incidence of epilepsy in developed societies has been found to be around 50 cases per 100,000 persons per year, and rises steeply in older age [16].

In pentelenetetrazole-induced seizure model, *N.oleander* 150 and 300 mg/kg produced significant ($p<0.01$) reduction in duration of convulsion and was comparable to that produced by diazepam(Table-2) . In the maximum electroshock induced seizure model, *N.oleander* 150 and 300 mg/kg and diazepam showed significant ($p<0.01$) reduction in duration of convulsion, but *N.oleander* 75 mg/kg did not exhibit anticonvulsant effect(Table-1). The anticonvulsant activity of *N.oleander* at various dose levels viz, 150 and 300 mg/kg p.o. were studied by the pentelenetetrazole and maximum electroshock-induced seizure models. The most popular and widely used animal seizure models are the traditional maximum electroshock-induced seizure and pentelenetetrazole tests. Prevention of seizures induced by pentelenetetrazole in laboratory animals is the most commonly used preliminary screening test for characterizing potential anticonvulsant drugs. The maximum electroshock-induced seizure test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures. By contrast, the pentelenetetrazole test represents a valid model for human generalized myoclonic and also absence seizures. Other chemoconvulsant models for primary generalized seizures include by bicuculine (GABAA receptor antagonized), strychnine (glycine receptor antagonist) and aminophylline (adenosinereceptor antagonist). The pentelenetetrazole assay has been used primarily to evaluate antiepileptic drugs. However, it has been shown that, most anxiolytic agents are also able to prevent or antagonize metrazoleinduced convulsion. Generally, compounds with anticonvulsant activity in the petitmal epilepsy are effective in pentelenetetrazole-induced seizure model [17]. Data from the study showed that the tonic convulsion produced by pentelenetetrazole was significantly delayed by *N.oleander*. The preliminary phytochemical investigation of different extracts of *N.oleander* revealed the presence of carbohydrate, glycosides, flavonoid, triterpenoid and steroids. The result of the current study indicate that the ethanol, aqueous and pet.ether extracts of *N.oleander* has delayed the onset of convulsions induction, and was able to decreased the durations of hind limb extension, clonus and stupor phases of MES induced convulsion as compared to control . The standard drug phenytoin in a dose of 25 mg/kg body weight provided 100 % protection and also significantly reduced the duration of stupor when compared to control. In other words the alcoholic and aqueous extracts is able to decreased the duration of hind limb extension (extensor phase), clonus and also the duration of stupor phase, which indicate the alcoholic extract does.

The data also show that diazepam antagonize the pentelenetetrazole-induced convulsion. According to be Sarro et al, pentelenetetrazole may be exerting its convulsive effect by inhibiting the activity of gamma amino butyric acid (GABA) at GABAA receptors [18] the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion respectively [19,20] . Phenobarbitone and diazepam have been shown to exert their antiepileptic effects by enhancing the GABA-mediated inhibition in the brain [21]. It is possible that diazepam and *N.oleander* antagonize pentelenetetrazole convulsion in this study by enhancing GABA neurotransmission. Since the *N.oleander* delayed the occurrence of pentelenetetrazole-induced convulsion, it is probable that it may be interfering with GABA aminergic mechanism(s) to exert its anticonvulsant effect.The maximal electroshock test is the most widely used animal model in antiepileptic drug discovery, because seizure induction is simple and the predictive value for detecting clinically effective antiepileptic is high [22]. The maximal electroshock test identifies agents with activity against generalized tonic clonic seizures using clinically established antiepileptic drugs [22]. The pharmacology of acute maximal electroshock dose not differs from the pharmacology of generalized tonic-clonic seizures in genetic models with choronic epilepsy, eg. Audiogenicseizure susceptible mice and rats or epileptic gerbils [23]. In addition to identifying drug activity against generalized tonic-clonic seizures, it has often been proposed that the maximal electroshock test predicts anticonvulsant drug effects against partial seizures. The anticonvulsant activity of *N.oleander* 300 mg/kg, in maximal electroshock model indicates that *N.oleander* might precipitate the tonic and clonic seizures.

Since inhibition of the MES test predicts activity against generalized tonic-clonic and cortical focal seizures so activity against MES induced seizures suggests that the ethanolic and aqueous extracts of N.oleander leaves are useful in suppressing generalized tonic-clonic seizures by regulating GABA mediated synaptic inhibition through

an action at distinct sites of this synopsis [24]. PTZ test predicts activity against absence seizures. Since PTZ is a GABAA receptor antagonist, the ethanolic and aqueous extracts may be acting by increasing GABA concentration in the brain.

CONCLUSION

The data obtained in present study indicated that ethenolic and aqueous extracts of *N.oleander* may be said to exert its anticonvulsant effect against MES- induced seizures via non-specific mechanisms. However more extensive study on mechanism of action and safety of the plant as medicinal remedy has to be carried out. The probable mode of action may be due to GABAaminergic mediation, glycine inhibitory mechanism and inhibit the electrical kindling effect. In conclusion, The precise mechanisms of possible anticonvulsant effect of plant extracts are not clear. Further research is in progress to isolate the compound responsible for this activity.

REFERENCES

1) McNamara JO. Drugs effective in the therapy of the epilepsies. In: Goodman and Gillman's The pharmacological basis of therapeutics. Hardman, JG, Limbird LE (eds). 10th ed. NewYork, McGraw-Hill, 2001, pp 521-39.
2) Delgado-Escueta AV, Treiman DM, Walsh GO. The treatable epilepsies. N. Engl J Med 1983; 308: 1508-1514.
3) WHO. Epilepsy: Etiology, epidemiology and prognosis.www.who.int/ entire/ mediacentre/factsheets/fs165/en/-25k-16jan2006.
4) Mattson RH. Drug treatment of partial epilepsy. Adv Neurol. 1992; 57: 643-50.
5) Gupta YK, Malhotra J. Adenosinergic system as an endogenous anticonvulsant mechanism. J Physiol Pharmacol. 1997; 41; 329-43.
6) Kirtikar K.R., Basu B.D.: Indian Medicinal Plants. International book distributors, second Edition, Vol. 3, 2005, 2220.
7) Neumann W. and Lindner W., Arch. Exptl.Path.Pharmakol., 1937; 185:630.
8) Nuki B., Folia.Pharmacol. Japan, 1949;45: 134.
9) GP Pendse, S Dutt, Chemical examination of the bark of Nerium odorum. Soland. Bull Acad Sci United Prov Agra and Oudh Allahabad India, 1934; 3: 209-14.
10) Kokate CK Practical Pharmacognosy Vallabh Prakashan: New Delhi, 1994, 56-58.
11) Khandelwal KR, Kokate CK, Purohit AP, Gokhale SB. Practical Pharmacognosy Techniques and Experiments Nirali Prakashan: Pune 2002, 157- 159.
12) Gupta YK, Malhotra J, George B and Kulkarni S.K. Methods and consideration for experimental Evaluation of antiepileptic drugs. Indian J Physiol Pharmacol.1999; 43 (1): 25-7.
13) Jesupillai et al; Pharmacologyonline, 2008; 3: 744-747
14) Vogel HG, Vogel WH. 1997. Drug Discovery and Evaluation, Pharmacological Assay Berlin: Springer.
15) Noel NW, Joseph AA, Helen OK, Steven SG, Asa A. Anti-seizure activity of the aqueous leaf extract of *Solanum nigrum* linn (solanaceae) in experimental animals. *African Health Sciences* 2008; 8(2): 74-79.
16) Marjan NA., Schwann SR., Farzaneh Z. Anticonvulsant effects of aerial parts of *Passiflora incarnata* extract in mice: involvement of benzodiazepine and opioid receptors. Biomed Central 2007;7:26. 1-6.
17) Loscher W, Schmidt D. Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. Epilepsy Res. 1988; 2; 145-81.
18) De Sarro A, Cecchetti V, Fravolini V, Naccari F, Tabarrini O, De Sarro G. Effects of novel β-desfluroquinolones and classic quinolones on pentelenetetrazole-induced seizures in mice. Antimicrob Agents Chemother. 1999; 43: 1729-36.
19) Meldrum BS. GABA agonists as antiepileptic agents. Advan Biochem Psycopharmacol. 1981; 26: 207-17.
20) Westmoreland BF, Benarroch EE, Dube JR, Regan TJ, Sandok BA. Medical neurosciences. Mayo Foundation Rochester 1994, pp 307-12.
21) Porter RJ, Meldrum BS. Antiseizure drugs. In: Basic and clinical pharmacology. Katzung BG (ed). New York, Lange Medical Books/McGraw-Hill, 2001, pp 403-17.
22) White HS. Clinical significance of animal seizure models and mechanism of action, studies of potential antiepileptic drugs. Epilepsia 1995; 38 Suppl: S9-17.
23) Loscher W. Animal models of epilepsy for the development of antileptogenic and disease modifying drugs: A comparison of pharmacology of kindling and poststatus epilepticus models of temporal lobe epilepsy. Epilepsy Res. 1999; 50: 105-23.
24) Swinyard A, Brown WC and Goodman LS. J.Pharmacol Exp Ther. 1952;106:319-330

Table-1: Effect of leaves of N.Oleander against MES induced convulsions

Sl.No	Treatment		Time (Sec) in various phases of convulsions (MEAN±SEM)				
	Drug	Dose (mg/kg)	Flexon	extensor	Clonus	Stuper	Recovery/Death
1	Normal Saline	5 ml/kg	3.2±0.15	17.33±1.7	16.6±2.2	102.5±2.8	Recovery
2	Phenytoin	25	1.4±0.12***	0	31.35±0.23	66.86±0.32***	Recovery
3	PEE	75	3.23±0.16	17.33±1.68	17.83±2.42	99.83±2.83	Recovery
		150	1.48±0.13**	16.33±1.38	23.51±2.29	94.16±9.12*	Recovery
		300	1.35±0.09***	14.66±1.2**	24.05±1.89	62±2.08***	Recovery
4	ALE	75	2.83±0.25*	16.16±1.6	17.16±2.18	112±2.91	Recovery
		150	1.58±0.16**	13.5±1.17**	15.01±1.26	90.16±6.76**	Recovery
		300	1.2±0.05***	9.48±0.48***	12.16±1.37	70.16±4.03**	Recovery
5	AQE	75	3.81±0.17	21.16±1.79	27.66±2.34	114.5±2.65	Recovery
		150	2.65±0.27**	15.8±1.9*	22.18±1.58	97.8±5.05*	Recovery
		300	2.53±0.18**	11.5±0.76**	18.96±1.27	64.5±3.73***	Recovery
Values are expressed as MEAN±SEM one way Anova followed by Dunnets 't' test Note: n=6 in each group, *P<0.05,**P<0.01,***P<0.001							

Table-2: Effect of leaves of N.Oleander against PTZ induced convulsions

Sl.No	Treatment		Onset of clonus	Onset of tonic	% Protection
	Drug	Dose (mg/kg)			
1	Normal Saline	5 ml/kg	84.16±3.6	381.6±7.36	00
2	Diazepam	5	0.7±0.35	Ab	100
3	PEE	150	122.6±7.37*	459.6±20.43**	50
		300	147.16±10.06**	570.83±49.58***	67
4	ALE	150	221.8±6.2***	540±30.24	67
		300	273.6±6.2***	764.6±32.89	84
5	AQE	150	124±4.7**	420.6±17.58**	50
		300	153±5.26**	531.6±26.8**	84
Values are expressed as MEAN±SEM one way Anova followed by Dunnets 't' test Note: n=6 in each group, *P<0.05,**P<0.01,***P<0.001					

Source of support: Nil, Conflict of interest: None Declared