INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIOMEDICAL ANALYSIS | ISSN: 2278 –2664
| OCT-DEC 2013| VOLUME 2 | ISSUE 4| 01-08
Available Online Through: www.ijprba.com

Research Article

EVALUATION OF ANTI-EPILEPTIC ACTIVITY OF ETHANOLIC EXTRACT OF *LANTANA CAMARA LINN.* IN MES AND PTZ INDUCED CONVULSIONS IN RATS

Krishna Mohan Chinnala*, Madhan Mohan Elsani, Himavanth Kumar K, Swetha Veldandi

Department of Pharmacology, St. John College of Pharmacy, Yellapur, Hasanparthy, Warangal-506371. Andhra Pradesh, INDIA.

***Corresponding author E-mail:** drchinnala@gmail.com

ABSTRACT

Title: Evaluation of anti-epileptic activity of ethanolic extract of *Lantana camara* Linn. in MES and PTZ induced convulsions in rats

Objective: To evaluate the antiepileptic activity of ethanolic extract of *Lantana camara* Linn. in albino Wistar rats.

Methods: In the present study ethanolic extract of *Lantana camara* Linn. was studied for its protective effect against maximum electroshock (MES) and pentylenetetrazole (PTZ) induced convulsions in Wistar rats. In MES method Seizures were elicited with a 60 Hz alternating current of 150 mA intensity for 0.2 sec. The Wistar rats were pretreated with ethanolic extract of *Lantana camara* Linn (EELC) for 14 days and standard group animals with Phenytoin (25mg/kg/i.p.). In PTZ induced convulsions method animals were pretreated with ethanolic extract of *Lantana camara* Linn. for 14 days and standard group animals with Diazepam (4mg/kg/i.p.) on 14th day PTZ (80mg/kg/i.p) was used as the inducing agent.

Results: It is found that treatment with ethanolic extract of *Lantana camara* Linn. (200 mg/kg and 400mg/kg) significantly ($p<0.001$) protected the animals especially hind limb tonic extensor (HLTE) stage in MES induced epilepsy. It is also found that EELC 200 mg/kg and 400 mg/kg had shown a significant ($p<0.001$) increase in onset of clonic convulsions comparable with standard treated animals.

Conclusion: The ethanolic extract of *Lantana camara* Linn. was showed significant ($p<0.001$) dose dependent protection in Wisar rats against MES and PTZ induced convulsions.

KEY WORDS

Antiepileptic activity, Latana camara, Convulsions, MES, PTZ.

INTRODUCTION

Epilepsy is the term used for a group of disorders characterized by recurrent spontaneous seizures that apparently result from complex processes involving several neurotransmitter systems such the glutamatergic, cholinergic, and gabaergic system[1]. According to the WHO, about 450 million people in the entire world have suffered mental, neurological, or behavioral problems at some time in their life and the prevalence rate for epilepsy are 1–2% of the world population[2]. The essential feature of the epilepsies is the appearance of behavioural changes, termed seizures. Such seizures are thought to occur via an alteration in the behaviour of neuronal

networks in the brain that induce the spontaneous expression of periods of synchronized burst firing interspersed by periods of normal electrical activity[3]. Glutamate and ɣ-amino butyric acid (GABA) are quantitatively the most important excitatory and inhibitory neurotransmitters, respectively, in the mammalian brain[4]. Thus, receptors for these two neurotransmitters are regarded as important targets for antiepileptic drugs. Despite the state-of-the-art medical treatment, drug-resistance remains a major clinical problem for one in three epileptic patients. Approximately 30% of patients with partial epilepsy and 25% of patients with generalized epilepsy are not well controlled on medications[5]. These patients often receive multiple medical treatments to control their seizures. Thus, there is an unmet need for new anti epileptic drugs. Herbal medicine could be a source for new therapeutics[6].

Lantana camara (verbenaceae) also known as "*SPANISH FLOG"*[7]. The native range of *Lantana camara* Linn. includes India, Mexico, Central America, and the Greater Antilles. The Bahamas, Colombia, and Venezuela. In the Kenyon highlands it grows in many areas that receive even minimal amount of rainfall. It can be seen in the wild and along footpaths, deserted fields and farms[8]. The parts of plants have been traditionally used for medicinal purpose. Extracts of the fresh leaves are antibacterial and are traditionally used in Brazil as an antipyretic, carminative and in the treatment of respiratory system infections. Plant pacifies vitiated vata, kapha, malaria toothache, wounds, ulcers, swelling, skin diseases, fistula, pustules and arthritis[9]. It is reported that the plant consists potential antitumor agents[10]. The authors reported the anti ulcerogenic activity of *Lantana camara* leaves on gastric and duodenal ulcers in experimental rats[11]. Present study was aimed to investigate the pharmacological effect of *Lantana camara* Linn. against antiepileptic activity by using Maximum Electro Shock (MES) and Pentylenetetrazole (PTZ) methods in Wistar rats.

MATERIALS AND METHODS

Plant collection

The aerial parts (leaves, stem, and fruits) of *Lantana camara* Linn. were collected during December 2010 from Kakatiya University, Warangal, Andhra Pradesh, India. It was identified and authenticated by Prof. Dr. Vatsaavaya S.Raju, Department of Botany, Kakatyia University, Warangal, Andhra Pradesh, India. The voucher specimen was maintained in our laboratory for the future reference.

Preparation of extract:

The aerial parts of plant was dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (500gm) 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. The ethanolic extract of *Lantana*

camara Linn. yield thick green semisolid residues (EELC, 25.8% w/w). The extracts were subjected to preliminary phytochemical screening and EELC revealed the presence alkaloids, flavonoids, carbohydrates, glycosides, tannins, terpeniods, and phenols[12].

Experimental animals:

Adult male Wistar rats, weighing 150-220g, were procured from the animal house of St. John College of pharmacy, yellapur, Warangal (Reg., no.1278/ac/09/CPCSEA). The animals were kept in polypropylene cages (6 in each cages) under standard laboratory condition (12 hr light and 12 hr dark day night cycle) and had free access to commercial pellet diet with water *ad libitum.* The temperature was maintained at 25 ± 1^{0}C with relative humidity (50 ±15%). The study was approved by the institutional animal ethical committee. Ethical norms were strictly followed during all experiments.

Acute oral toxicity study:

The acute toxicity of 90% ethanolic extract of *Lantana camara* Linn. (EELC) was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study[13]**.**

Anti-Epileptic Activity:

Maximal electroshock seizures (Mes) induced convulsions:

Adult male Wistar rats weighing 150 to 220 gm were randomized and divided into four groups of six animals each (*n*=6).

Group-I: Control: Animals received vehicle (1% w/v SCMC, 1ml/100 g).

Group-II: Animals received standard drug (Phenytoin, 25mg/kg) intraperitoneally.

Group-III & IV: Animals received EELC (200 and 400 mg/kg/day, p.o.) respectively for 14 days.

On the 14th day, Seizures are induced to all the groups by using an Electro Convulsiometer. Maximal electroshock seizures were elicited by a 60 Hz alternating current of 150 mA intensity for 0.2 sec[14]. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes prior to application to the rats. This increases the contact and reduces the incidence of fatalities. The duration of various phases of epilepsy (Flexion, Extensor, Clonus, Stuper, and Recovery) were observed[15]. The percentage protection was estimated by observing the number of animals showing abolition of Hind Leg Tonic Extension (or) extension not greater than 90°**.**

Pentylenetetrazole (PTZ) Induced convulsions:

Adult male Wistar rats weighing 150 to 220 gm were randomized and divided into four groups of six animals each (*n*=6).

Group-I: Control: Animals received vehicle (1% w/v SCMC, 1ml/100 g).

Group-II: Animals received standard drug (Diazepam, 4mg/kg) intraperitoneally.

Group-III & IV: Animals received EELC (200 and 400 mg/kg/day, p.o.) respectively for 14 days. On the 14th day, Pentylenetetrazole (PTZ) (90mg/kg body weight, *s.c.*) was administered

to all the groups to induce clonic convulsions[16]. Animals were observed for a

period of 30mins post – PTZ administration. The parameters noted were mean onset time of convulsions, duration of convulsion and recovery/Death (% recovery or % of survival) due to PTZ[17].

RESULTS

Effects of EELC on MES Induced Convulsions

Phenytoin treated animals have shown 100% protection against MES induced seizures where as EELC 200 mg/kg and 400 mg/kg have shown 68.88% and 83.66% protection respectively against MES induced seizures. The EELC at both doses and standard treated animals had shown a significant change in duration at all stages of convulsions. EELC 200 mg/kg and 400 mg/kg had shown a significant decrease in the duration of tonic extensor phase and comparable significance ($p<0.001$) with the control and standard animals. The results were shown in **Table.1 and Figure.1.**

Effects of EELC on MES Induced Convulsions

Diazepam treated animals have shown 100% protection against PTZ induced seizures where as EELC 200 mg/kg and 400 mg/kg have shown 69.97% and 81.5% protection respectively against PTZ induced seizures. EELC 200 mg/kg and 400 mg/kg had shown a significant increase in onset of clonic convulsions and comparable ($p<0.001$ and $p<0.001$) with the control and standard animals. The results were shown in **Table.2 and Figure. 2.**

Table 1: Effect EELC on MES Induced Convulsions

Groups	Drug Treatment	Percentage Protection	Flexion (Sec)	Extensor (Sec)	Clonus (Ssec)	Stupor (Sec)	Recovery (Sec)
I	Control (1% w/v SCMC)	0	6.64±0.26	13.4±0.84	15.20±1.24	30.86±0.82	162.40
II	Phenytoin (25 mg/kg/i.p.)	100	3.46±0.46 ^{a***}	0	10.2±1.45 ^{a***}	12.26±0.45 ^{a***}	112.64
III	EELC (200 mg/kg/p.o.)	68.88	4.68±0.34 ^{b***}	4.17±0.16 ^{b***}	7.44±0.26 ^{b***}	18.34±2.34 ^{b***}	146.20
IV	EELC (200 mg/kg/p.o.)	83.66	3.88±0.38 ^{b***}	2.19±0.24 ^{b***}	5.62±0.42 ^{b***}	15.24±1.72 ^{b**}	122.46

Values are mean ± SEM of six (n=6) observations
Comparison between: a- Group I and Group II, b- Group II Vs Group III, Group IV.
Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test.
*p<0.05; ** p<0.01; ***p<0.001; ns-non significant.

Figure. 1: Effect of EELC on MES induced epilepsy

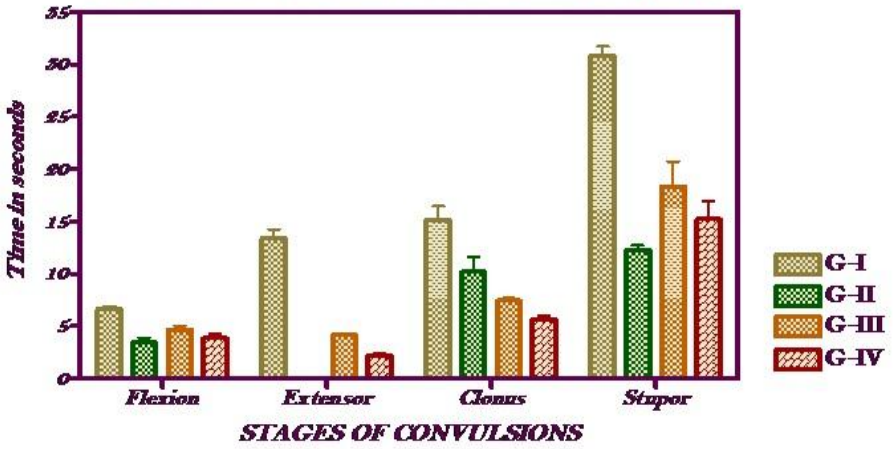
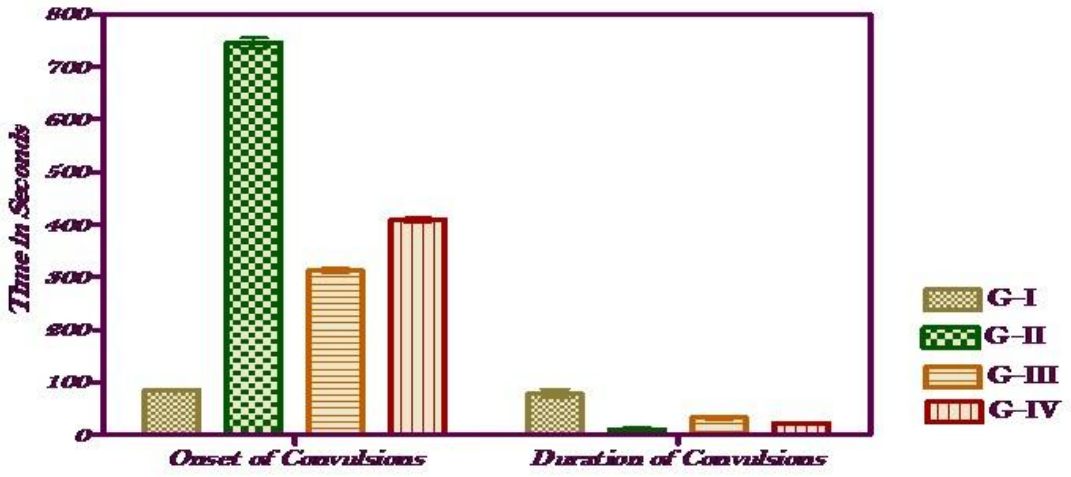


Table 2: Effect of EELC on PTZ induced epilepsy

Groups	Treatment	% of Protection	Onset of Clonic convulsions	Duration of Convulsions
I	Control (1% w/v SCMC)	0	84.64±1.560	78.29±8.830
II	Diazepam (4mg/kg/i.p.)	100	745.30±9.320 a***	10.23±2.612a***
III	EEL (200 mg/kg/p.o.)	58.04	312.60±3.810b***	32.85±3.307b***
IV	EELC (200 mg/kg/p.o.)	71.07	408.70±3.676b***	22.63±1.260b***

Values are mean ± SEM of six (n=6) observations
Comparison between: a- Group I and Group II, b- Group II Vs Group III, Group IV.
Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test.
*p<0.05; ** p<0.01; ***p<0.001; ns-non significant.

Figure. 2: Effect of EELC on PTZ induced epilepsy



DISCUSSION

We have used the most popular two different animal models experiments such as MES and PTZ methods which characteristically described three types of seizures activity[18]. The MES test is the most frequently-used as an animal model for identification of anticonvulsant activity of drugs for the generalized ("grand mal") tonic-clonic seizures and partial seizures[19,20]. PTZ-induced seizures test is considered as an experimental model for the "generalized absence seizures" and also a valid model for human generalized myoclonic seizures and generalized seizures of the petitmal type[21].

In our present study, it is found that treatment with EELC on rats significantly protected the animals especially hind limb tonic extensor (HLTE) stage in MES induced epilepsy. The current antiepileptic drugs (AEDs) that are clinically effective in the management of grandmal epilepsy such as Phenytoin, carbamazipine, primidone, valproate and lamotigine all suppress the HLTE in MES induced seizures[22]. Protection against HLTE also indicates the ability of a testing material to inhibit or prevent seizures discharge within the brainstem seizure substrate[23]. The ability of the extract to inhibit the HLTE in MES test as compared to Phenytoin (100% protection) in the model suggests anticonvulsant activity for the management of generalized tonic-clonic and partial seizures; it suggests the presence of anticonvulsant compounds in EELC.

Similarly, we found that treatment with EELC on PTZ induced rats significantly reduce the duration of convulsion and delayed the onset of clonic convulsion. AEDs effective in the therapy of generalized seizures of (absence or myoclonic) petit mal type such as benzodiazepines, barbiturates and vigabatrine exhibit dose dependent suppression of various seizure pattern induced by PTZ[24]. It was known that PTZ may be exerting its convulsant effect by interfering the activity of inhibitory neurotransmitter gamma amino butyric acid (GABA) at $GABA_A$ receptor[25]. The antagonism of PTZ- induced seizures suggests the interaction of the EELC with the GABA-ergic neurotransmission.

From the above observation it could be predicted that the ability of the extract to exhibit activity against these two types of seizures suggests that it may act through different mechanisms to elicit its anticonvulsant effects, such as inhibition of voltage-gated sodium channels or by enhancing the GABAergic pathway. Further studies to establish the active chemical constituent(s) of the extract and the exact mechanism of action is currently going on in our laboratory.

REFERENCE

1. Sander JW, Shorvon SD. Epidemiology of the epilepsies. *J. Neurol. Neurosurg. Psychiat.* 1996, *61*, 433-443.
2. WHO. *The World Health Report. Mental Health: New Understanding New Hope*; WHO: Geneva, Switzerland, 2001.
3. Dichter M. Basic mechanisms of epilepsy: targets for therapeutic intervention. *Epilepsia* 1997; 38 (Suppl. 9): S2– S6.
4. Rang HP, Dale MM, Ritter JM, Moore PK, Pharmacology. Churchill Livingstone, Edinburgh 2007.
5. Richens A, Perucca E. Clinical pharmacology and medical treatment. In: Laidlaw J, Richens A, Chadwick, D. (Eds.), A Textbook of Epilepsy. Churchill Livingstone, Edinburgh 1993: 495–560
6. Mikael E. Pedersen, Henrik T. Vestergaard, Suzanne L. Hansen, Sekou Bah, Drissa Diallo, Anna K. Jäger. Pharmacological screening of Malian medicinal plants used against epilepsy and onvulsions. *Journal of Ethnopharmacology* 2009; 121: 472–475.
7. *Germplasm Resources Information Network*. United States Department of Agriculture. 2007: 05-29.
8. Habitat and Range www.richfarmgarde.com
9. Duke James A. Handbook of phytochemical constituents of GRAS herbs and other economic plants. Boca Raton, FL. CRC Press; 1992.
10. Shashi BM, Niranjan PS, Subodh KR, Sharma OP. Potential Antitumor Agents fromf *Lantana camara*: Structures of Flavonoid and Phenylpropanoid Glycosides. Tetrahedron. 1994; 50:9439–9446.
11. Thamotharan G, Sekar G, Ganesh T, Saikat sen, Raja chakraborty, Senthil kumar N. Anti-ulcerogenic effects of *Lantana camara* linn. leaves on in vivo test models in rats.
12. Jayaraman J. *Laboratory Manual in Biochemistry*. New age International (P) Ltd, 1st Edition 1981: 51.
13. OECD, 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals organisation for economical co-operation and development, Paris, June, 2000.
14. Kulkarni SK, *Handbook of Experimental Pharmacology*. Vallabh Prakashan, 3rd Edition 1999: 131-134.
15. Ambawade SD, Kasture VS, Kasture SB, Anticonvulsant Activity of Roots and Rhizomes of *Glycyrrhiza glabra*. *Indian journal of pharmacology* 2002; 34: 251-255.
16. Rao VS, Anjali Rao and Sudhakar k. Anticonvulsant and neurotoxicity profile of *Nardostachys jatamasi* in rats. *Journal of Ethnopharmacology* 2005; 102: 351-356.
17. Patil KS, Suresh babu AR and Chaturvedi SC.Anti-convulsant activity of roots and barks of *Calotropis gigatea* Linn. *Journal of Natural Remedies* 2008; 8(1): 109-114.
18. Ya'u J, Yaro AH, Abubakar MS, Anuka JA, Hussaini IM. Anticonvulsant activity of *Carissa edulis* (Vahl) (Apocynaceae) root bark extract. *Journal of Ethnopharmacology* 2008; 120: 255-258.
19. 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.
20. Delgado JN, Remers WA(Eds.). Wilson and Gisvold's Textbook of Organic and Medicinal Pharmaceutical Chemistry. Lippincottt-Raven, U.S.A. 1998.
21. Oliveira FA, Almeida RN, Sousa MFV, Barbosa-Filho JM, Diniz SA, Medeiros IA. Anticonvulsant properties of *N*-salicyloyltryptamine in mice. *Pharmacol Biochem Behav* 2001; 68:199-202.
22. Rho JM, Sankar R. The pharmacological basis of antiepileptic drug action. Epilepsia 1999; 40: 1471-1483.
23. Browing R. The electroshock model, neuronal network and antiepileptic drugs. In: Faingold, CL, Fromm GH (Eds), Drugs for control of epilepsy: Actions on neural networks in seizure disorder. CRC Press, Boca Raton FL 1992: 195-211.
24. Loscher W, Honack D, Fassbender CP, Nolting B. The role of technical biological and pharmacological

factors in the laboratory evaluation of anti convulsant drugs. *Epilepsy Reaserch* 1991; 8: 171-189.

25. Quintans-Junior LJ, Souza TT, Leite BS, Lessa NMN, Bonjardim LR, Santos MRV, Alves PB, Blank AF, Antoniolli AR. Phytochemical screening and anticonvulsant activity of Cybopogon winterianus Jowitt (Poaceae) leaf essential oil in rodents. Phytomedicine 2008; 15: 619-624.