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Anticonvulsant Activity of Ethanolic Extract of Clerodendrum infortunatum linn in Rats

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ABSTRACT: Clerodendrum infortunatum Linn. (Verbenaceae) is an important and widely used medicinal plant in Indian folk medicine in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation and epilepsy. This study evaluates the anticonvulsant effect of the ethanolic extract of the leaves of Clerodendrum infortunatum Linn. (EECI) in experimental animals. Acute toxicity test for the EECI was done according to the Organization for Economic Co-operation and Development (OECD) guidelines. Following the Phytochemical analysis of the plant extract for specific plant constituents, the anticonvulsant effect of EECI (100mg/kg, 200mg/kg and 300mg/kg b.w i.p) was evaluated against Maximum electroshock (MES) induced convulsions using phenobarbitone (10mg/kg b.w) as the reference drug. EECI (400mg/kg b.w i.p) significantly delayed ($p<0.01$) the onset and antagonized MES-induced seizures. The anticonvulsant activity exhibited by the plant extract could be attributed to the saponin constituent. Clerodendrum infortunatum Linn. as a commercial source of anticonvulsant drug needs to be subjected to further research.

Keywords: Clerodendrum infortunatum; Anticonvulsant; Phenobarbitone; Maximum electroshock and Convulsion.

INTRODUCTION: Epilepsy are a group of disorders of the CNS characterized by paroxysmal cerebral dysrhythmia, manifesting as brief episodes (seizures) of loss or disturbance of consciousness with or without characteristic body movements (convulsions), sensory or psychiatric phenomena.[1] It is a chronic neurological disorder that affects people of all ages. Around 0.5-1% of the world's population are affected with epilepsy and 30,000 people develop epilepsy every year.[2 & 3] In India studies have reported the prevalence rate of epilepsy varying from 1720 to 9800 cases per million population. The research for perfect antiepileptic compound with more selective activity and lower toxicity continues to be an area of intensive investigation in medicinal chemistry. Moreover many side effect are reported in many patient treated with present available antiepileptic drugs (AEDs).[4]

Clerodendrum infortunatum Linn. (Verbenaceae) is an important and widely used medicinal plant, reported to contain active bitter substance like clerodin, has been widely used as tonic and anithelmintic agent in the country sides of North India. Though, variously used in Ayurveda, Unani system of medicine and Homeopathy in case of ailments like diarrhoea, skin disorders, venereal and scrofulous complaints, wounds, post-natal complications, as external applications on tumours, etc., the plant needs thorough investigation for its specific medicinal activity. Leaves of the plant are prescribed for tumour, certain skin diseases and scorpion sting. Pharmacological actions include Antimicrobial, antioxident, analgesic, anticonvulsant and antipyretic activities. The antioxidant, antimicrobial, anti-malaria, anthelmintic and analgesic activities of the plant have further created an upsurge in investigations on the plant.[5 & 6] This study was undertaken to evaluate the analgesic, anti-inflammatory, anticonvulsant activity of ethanolic extract of Clerodendrum Infortunatum Linn in experimental animals.

MATERIAL AND METHODS:

1. MATERIALS:

1.1 Leaves of Clerodendrum infortunatum Linn.: Fresh leaves of C. infortunatum Linn. were collected from Amrutha Vana Centre for Herbal Gardens and Landscaping Services, Govt. of Karnataka, Bangalore, in the month of August 2012. The plant identity was authenticated by botanist Prof. Jadimath.

1.2 Ethanolic extract of leaves of Clerodendrum infortunatum.

1.3 Wistar albino rats of either sex: All the animals were obtained from the Animal house, Department of Pharmacology, S N Medical College, Bagalkot. Wistar albino rats of either sex weighing 150-250g were se-

lected for the experiment. Pregnant rats, animals with infection, injuries, deformities and any other abnormalities were excluded from the study. All the animals were maintained at 12: 12 hr dark: light cycle, 25±2°C, and 35%-60% humidity. All the animals were received standard laboratory diet (VRK nutritionals, Pune) and water was provided ad libitum.

1.4 Phenobarbitone: (10mg/kg b.w): Phenobarbitone (barbiturate) is a broad spectrum anticonvulsant effective against generalized tonic-clonic, simple partial and complex partial seizures, [1] which has been used as the standard drug against which the anticonvulsant activity of the plant extract was tested in experimental animals.

1.5 Electroconvulsiometer: This instrument is used to induce convulsions experimentally which are hypothesized to originate from forebrain or brainstem. Electrical stimulation via ear electrodes is provided using stimulation parameters as 150mA for 50-60 /sec for 0.2sec, with constant voltage of 750V for rats.

1.6 Soxhlet apparatus: Consists of a Soxhlet extractor, used to obtain the Ethanolic extract of leaves of Clerodendrum infortunatum.

2. METHODS:

2.1 Preparation of extract: The leaves of the Clerodendron infortunatum were dried under shade for a period of four weeks. The dried plant material was milled to a fine powder using the mechanical grinder. The powder plant material was extracted with absolute ethyl alcohol using Soxhlet extraction apparatus. Dried powder (300 g) was extracted in a Soxhlet extractor with ethanol for about 8-9 h at 45ºC. Extract was collected and dried using rotary flash evaporator at 40-45ºC and crude residue was collected. The solvent was completely removed under reduced pressure and semisolid mass was obtained. The yield was calculated as 30 g. The extract was stored in well closed glass container at 5ºC in refrigerator for further study.

2.2 Acute oral toxicity study: It was done according to Organization for Economic Co-operation and Development (OECD) guidelines 425 (up and down procedure). All the five mice were administered 2000mg/kg of ethanolic extract of leaves of Clerodendrum infortunatum orally and observed continuously for a period of 14 days, every hourly for 24 hours, and every day for 14 days for its movements, grooming activity, exploring activity, writing reflex, eye movements, and convulsion etc.[7]

2.3 Phytochemical analysis: Qualitative Phytochemical Analysis of Plant Extracts: The leaf extracts were analyzed for flavonoids, alkaloids, glycosides, saponins, tannins, proteins and aminoacids, sterols and triterpenoids, carbohydrates, fixed oils, anthraquinone, steroids and resins.

i) Flavonoids: Alkaline reagent test: To the test solution, few drops of sodium hydroxide solution was a; formation of an intense yellow color, which turns to colorless on addition of few drops of dilute acid, indicates presence of Flavonoids.

ii) Alkaloids: Tannic acid test: Alkaloids give buff color precipitate with 10% Tannic acid solution.

iii) Glycosides: Keller killiani test: 0.4ml of glacial acetic acid containing a trace amount of ferric chloride was added to the extract and a small amount transferred to a small test tube. Add carefully 0.5ml of concentrated sulphuric acid to the side of the test tube, blue color appears in the acetic acid layer.

iv) Saponins: Froth Test: I ml solution of drug in water is placed in a semi-micro tube and shaken well and noted for a stable froth.

v) Tannins: Ferric chloride test: Test solution gives blue green color with ferric chloride.

vi) Proteins and aminoacids: Millon's test: Test solution with 2ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid), white precipitate appears, which turns red upon gentle heating

vii) Sterols and Triterpenoids: Libermann-Bachard test: Extract is treated with few drops of acetic anhydride, boiled and cooled. Conc. sulfuric acid is added from the sides of the test tube. Formation of a brown ring at the junction of two layers is seen. If the upper layer turns green indicates the presence of steroids and formation of deep red color indicates the presence of triterpenoids.

viii) Carbohydrates: Benedict's test: Treat the test solution with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex). Boil on water bath. Reddish brown precipitate forms if reducing sugars are present.

ix) Anthraquinones: Borntragers Test: About 0.5g of the extract was taken into a dry test tube and 5ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate was shaken with equal volume of l0% ammonia solution. A pink violet or red colour in the ammonical layer was observed for the presence of anthraquinone.

x) Resin: Five milliliter of distilled water was added to the extract and observed for turbidity.

xi) Steroids: Two milliliter of acetic anhydride was added to 0.5g of extract and 2ml of H_2S0_4 was added along the sides of the test tube and the result was observed for red colour ring formation.[8 & 9]

2.4 Evaluation of Anticonvulsant activity:

Maximum electroshock (MES)–Induced Seizures: Electroconvulsive shock, inducing Hind Limb Tonic

Extension (HLTE) in 99.9% of animals (Pourgholami et al, 1999) [50]. All the animals were given electrical stimulation through ear electrodes using Electro Convulsiometer; stimulation parameters included 150mA at 50-60 /sec for 0.2sec, with constant voltage of 750V for rats. Period of tonic hind limb extension was noted.

Animals (30 albino rats of either sex weighing 150-200 g) were randomly divided into five groups each consisting of six animals (n=6), each animal given single dose as 400mg/kg (Group II), 600mg/kg (Group III), 800mg/kg (Group IV). Groups divided as follows:

Group I: Served as control Normal saline 0.5ml (10ml/kg orally)

Group II: Served as standard Phenobarbital (10mg/kg b.w) and Maximum electroshock (150mA, 50Hz for 0.2 sec)

Group III: Ethanolic extract dose (400mg/kg) and Maximum electroshock (150mA, 50Hz for 0.2 sec)

Group IV: Ethanolic extract dose (600mg/kg) and Maximum electroshock (150mA, 50Hz for 0.2 sec)

Group V: Ethanolic extract dose (800mg/kg) and Maximum electroshock (150mA, 50Hz for 0.2 sec)

2.5 Statistical analysis: All results are expressed as the mean ± SEM. The results were analyzed for statistical significance ($p < 0.05$, $p < 0.01$) by one-way (ANOVA) followed by Dunnett's test using computerized Graph Pad lnStat version 3.05. Graph pad software. U.S.A.

Figure 1: Clerodendrum infortunatum Linn.

Figure 2: Rat showing convulsive activity by MES method using ear electrodes.

RESULTS AND DISCUSSION:

Maximum electroshock (MES)–Induced Seizures method for evaluating anticonvulsant activity: Anticonvulsant activity was evaluated using the Maximal electroshock seizure test with Phenobarbital as the standard.

The maximal electroshock seizure test, in which tonic hind limb seizures are induced by bilateral corneal or transauricular eleccrical stimulation, is thought to be predictive of anticonvulsant drug efficacy against generalized tonic-clonic seizures.[10]

MES and PTZ tests provide some insight into the ability of a given drug to penetrate the blood-brain barrier and exert a central nervous system (CNS) effect. Indeed, both models are nonselective with respect to mechanism and therefore arc well suited for screening anticonvulsant activity, as neither model assumes that the pharmacodynamic activity of a particular drug is dependent on its molecular mechanism of action.[10] Drugs likely to be effective in Grandmal epilepsy usually confer protection against electrically induced convulsion in animals.[11]

In the present study, in each phase of MES-induced convulsions; i.e. Flexion, Extension, Clonus, Stupor, Recovery, there is significant reduction in time with the test drug (EECI) at doses 200 & 400 mg/kg which is comparable with that of the standard drug, phenobarbitone (10mg/kg). The p value obtained was significant (<0.01) and the percentage of protection of test drug at 200mg/kg body weight was 82.6% and at 400 mg/kg was 81.9%, which is comparable with the percentage protection of the standard drug, phenobarbitone at 10mg/kg body weight (100%). (Table 1 and Graph 1)

To conclude, EECI (400mg/kg b.w i.p) significantly delayed ($p<0.01$) the onset and antagonized MES-induced seizures.

The ethanolic extracts of the plant was found to possess statistically significant anticonvulsant activity ($p<0.01$) against Maximum electroshock (MES) – Induced Seizures. The findings are similar to previous studies by S.Das et al (2010)[94] and Pal Dilipkumar et al (2009).[12]

The significanant anticonvulsant effect of EECI could be due to the saponin constituents of the leaves as has been shown in previous studies; Saponin decreased the duration of seizures and gave protection in a dose dependent manner against leptazol-induced convulsions which suggest that saponin has significant anticonvulsant effect.[5 & 13] The anticonvulsant effects of are possibly mediated by chloride channels of GABA benzodiazepine receptor complex and by chloride

channel of glycine receptor.[14] Preliminary phytochemical screening of the plant extract exhibited the presence of flavonoid, alkaloids, tannin, saponins, sterols, and fixed oils.

In earlier studies Clerodendrum infortunatum Linn. leaves on preliminary chemical analysis were found to contain saponin, clerodin (a bitter diterpene) 4, 6 and some enzymes. Leaves also contain a fixed oil which consists of Glycerides of Lenoleic, oleic, stearic and lignoceric acid.Luperol and β-sitosterol from roots.Clerosterol identified as 5, 25- sigmastadien_3β-ol, clerodolone as lup_20 (30)-en- 3β-diol-12-one and clerodone as 3β-hydroxylupan- 12-one and a steroidal glycoside from roots.[5]

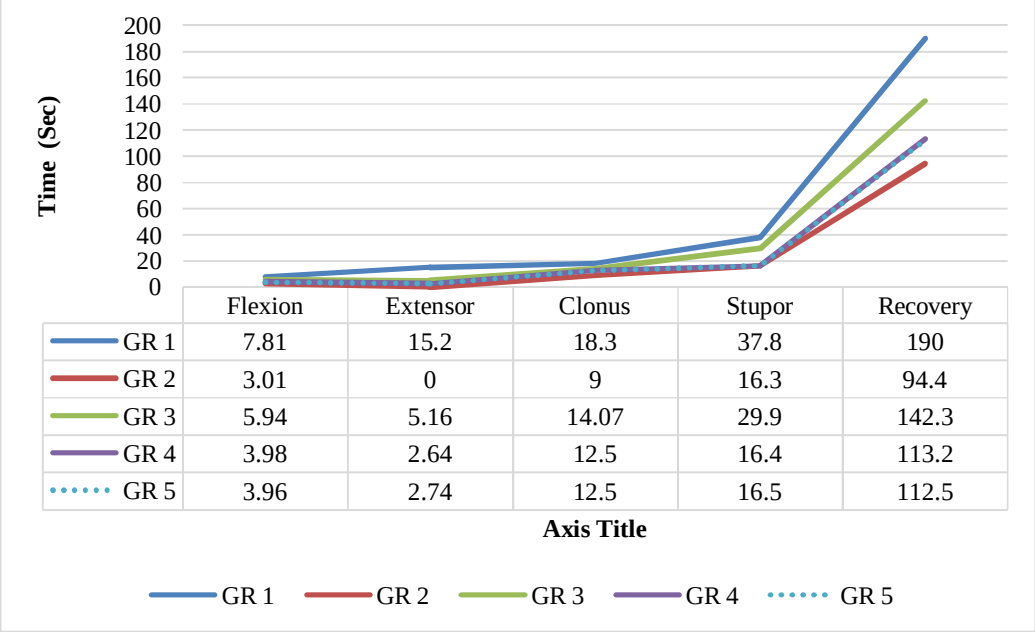
Previous phytochemical investigation of the plant revealed the presence of alkyl sterols and 2,-(3, 4-dehydroxyphenyl) ethanol 1-O-α-2 rhamnopyranosyl-(1→3)-β-D-(4-O-caffeoyl) glycopyranoside (acteoside) in this plant.[6.] The plant was also found to contain triterpenes, steroids and flavonoids.[5]

The presence of bioactive constituents, including flavonoids, is thought to promote the wound-healing process due to their antioxidant and antimicrobial activities. The hepatoprotective activity of the methanolic extract of Clerodendrum infortunatum may due to the presence of flavonoids, terpenoids and saponins in the extract.[5] Saponin might be attributed to the presence of anticonvulsant activity.

Table 1: Time duration (s) of phases of MES- induced convulsions.

Group	Flexion M ± S	Extension M ± S	Clonus M ± S	Stupor M ± S	Recovery M ± S	Percentage protection (%)
Control	7.81±0.77	15.2± 1.57	18.3±0.61	37.8±1.63	190 ±10.6	0
Standard (10mg/kg)	3.01±0.27	0	9 ± 0.74	16.3±1.42	94.4 ± 3.7	100
Test (100mg/kg)	5.94±0.59	5.16± 0.46	14.07±0.92	29.9± 1.6	142.3±4.4	66.05
Test (200mg/kg)	3.98±0.12	2.64± 0.31	12.5± 0.62	16.4±0.67	113.2±4.3	82.6
Test (400mg/kg)	3.96±0.14	2.74± 0.37	12.5± 0.56	16.5±0.65	112.5±3.4	81.9
F	104.4	357.3	138.1	364.6	235.8	-
d*f*	4	4	4	4	4	-
p	0.0001	0.0001	0.0001	0.0001	0.0001	-

M ± S: Mean ± Standard



GR: Group: - 1= Control; 2= Standard; 3= Test (100mg/kg); 4= Test (200mg/kg); 5= Test (400mg/kg)

Graph 1: Showing time duration (in seconds) of phases of MES induced convulsions.

CONCLUSION: In the present study, the extract from Clerodendrum infortunatum Linn. exhibited significant anticonvulsant activity in experimental animals. The present study also substantiates the traditional use of C.infortumatum Linn. for the treatment of various inflammatory ailments. The plant can be recommended for the further studies to isolate the active ingredients.

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REFERENCES:

1. K. D. Tripathi (2009) Essentials of Medical Pharmacology, (Jaypee Brothers Medical Publishers, Ed 6, 401-410.

2. Karan Singh and Bhavana verma. (2012) An ayurvedic insight towards epilepsy, *IJRAP*, 3(5), 682-689.

3. Babu Harish B., S. Mohana Lakshmi, Kumar Saravana A. (2010) A review on traditional system of medicine for treats Epilepsy, *International Journal of Biological & Pharmaceutical Research*, 1(1), 1-6.

4. A. G. Nikalje, A Altamash, M. S. Ghodke (2012) Herbal Anticonvulsant Agents: A Brief Review, *IJRPS*, 2(3):1-13.

5. Bhattacharjee Debayan, Das Arup et al. (2011) Clerodendrum Infortunatum Linn.: A Review, *Journal of Advances in Pharmacy and healthcare Research*, 1(3), 82-85.

6. Sudipta Das, Pallab K, Haldar, Goutam Pramanik and R.B. Suresh (2010) Evaluation of Anti-Inflammatory Activity of Clerodendron infortunatum Linn. Extract in Rats, *Global Journal of Pharmacology*, 4 (1), 48-50.

7. Maniyar Y, Bhixavathimath P. (2012) Antihyperglycemic and hypoglycemic activities of aqueous extract of Carica papaya Linn. Leaves in alloxan-induced diabetic rats, *Journal of Ayurveda and Integrative Medicine*, 3(2), 70-4.

8. Manjamalai A., Singh S. S., Guruvayoorappa C., Grace V. M. B. (2010) Analysis of phytochemical constituents and anti- microbial activity of some medicinal plants in Tamilnadu, India, *Global Journal of Biotechnology & Biochemistry*, 5(2), 120-128.

9. De S., Dey Y. N., Ghosh A. K. (2010) Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of Amorphaphallus paeonifolius (Araceae), *International Journal on Pharmaceutical and Biomedical Research*, 1(5), 150-7.

10. Lucindo J. Quintans Junior et al. (2008) Plants with anticonvulsant properties- a review, *Brazilian Journal of Pharmacognosy*, 18(Supl.), 798-819.

11. Jayarama Reddy, Gnanasekaran D., Vijay D., Ranganathan T.V. (2010) In vitro studies on anti asthmatic, analgesic and anti convulsant activities of the medicinal plant Bryonia laciniosa.Linn., *International Journal of Drug Discovery*, 2(2), 1-10.

12. Dilipkumar Pal, Santanu S., Kanti U. Mazumder (2009) Analgesic and anticonvulsant effects of saponin isolated from the leaves of Clerodendrum infortunatum Linn. in mice, *Indian Journal of Experimental Biology*, 47, 743-747.

13. Mittal Payal, Gupta Vikas, Kaushik Dhirender, Ghaiye Pankaj (2013) Medicinal plants used as anti-epileptic-an update, *Recent Developments on Neurological Diseases*, 129-154.

14. Sudipta Das, Pallab K, Haldar, Goutam Pramanik and R.B. Suresh (2010) Anticonvulsant activity of methanolic extract of Clerodendron infortunatum Linn. in Swiss albino mice, *Thai J. Pharm. Sci.*, 34, 129-133.