Research Article Available online www.ijrpsonline.com ISSN: 2249–3522

International Journal of Research in Pharmacy and Science

Anticonvulsant and Sedative Effects of Leaf Extract of *Gymnosporia emerginata*

K Hemamalini[1*], Uma Vasireddy[2], E Rathna sundari[2]

[1]Dept. of Pharmacology, TRR College of Pharmacy, Meerpet, Hyderabad, AP, India
[2]Dept. of Pharmaceutics, Dhanavanthri College of Pharmacy, Kothagudem, Hyderabad, AP, India

ABSTRACT

The herbal preparation of the leaves of *Gymnosporia emerginata* is used in traditional medicine for the treatment of Epilepsy and febrile convulsions. A pilot study has confirmed the potency of the leaf of *Gymnosporia emerginata* in the control of seizure in mice. The current study was aimed to identify the active phytochemical responsible for the activity. Results indicated that methanolic extract of *Gymnosporia emerginata* at 300 mg/kg exhibited a significant $P<0.05$ delay in the latency of myoclonic spasms and tonic-clonic phase of seizure induced by PTZ, and also decreased the latency and increased the duration of phenobarbitone induced sleeping time. While the phytochemical studies showed the presence of alkaloids, resins, glycosides, carbohydrates, reducing sugar, fats and oils, flavonoids and terpinoids. The results suggested that the extract possessed anticonvulsant activity and central depressant effects which may be attributable to the flavonoids.

KEYWORDS: *Gymnosporia emerginata*, Flavonoids, Sedative, Anticonvulsant

***Corresponding author:**
Dr. K. Hemamalini
H.O.D. Pharmacology, Dept. of Pharmacology,
TRR College of Pharmacy, Meerpet, Hyderabad
rkhemamalini@gmail.com

INTRODUCTION

The use of herbal preparations in the management of various forms of epilepsies is very common in many parts of the world. Epilepsy affects more than 50 million persons world wide[1]. Seizure is a characteristic feature in epilepsy and is associated with disordered and rhythmic high frequency discharge of impulses by a group of neurons in the brain and status epileptics is characterized by repeated episodes of epilepsy without the patient having recovered from the previous attack[2]. There are many classes of anticonvulsants that are of clinical usefulness with good prognosis for controlling seizures in most patients[3]. Despite this many patients have seizures that are not adequately managed by the established antiepileptic drugs[4]. Moreover, the high incidence of detestable adverse effects from the use of antiepileptic drugs is also a source of wide spread concern in patients who use them chronically. These and the treatment cost have made traditional herbs and herbalists very useful and indispensable in the struggle for seizure management and future antiepileptic drug development. There is therefore the need for research into medicinal plants with possible anticonvulsant effects, based on folklore use. *Gymnosporia emerginata*, belong to the family clasteraceae[5] and commonly used in India by the tribal people for the treatment of liver disorders, diarrhoeal diseases and cancer disorders. *Gymnosporia emerginata* leaves contains flavonoids, tannins and used as analgesic and wound healing property.

MATERIALS AND METHODS

The pharmacological work has been planned after the literature review from the literature the dose was selected for the animals were acclimatized to room temperature (280+ 50C) with relative humidity of 550+ 50% in a standard wire meshed plastic cages for 4 to 5 days prior to common cement of the experiment. All animals were fed standard animal feed and tap water adlibitum before the experiments. The animals were maintained as per the norms of CPCSEA (Regd.No.1447/PO/a/11/CPCSEA) and cleared by CPCSEA and institutional ethics committee (Teegala Ram Reddy College of Pharmacy) each experimental group consisted of five animals housed in separate cages

Plant material

The leaf of *Gymnosporia emerginata* plant was collected from local region of Trupathi, District A.P, and India in the month of June, 2010. The botanical identify was confirmed by a batanist Dr.K. Madhavachetty, S.V.University.

Preparations of the extracts:

5 kg of the leafs was made in to coarse powder and passed through seive. The seived powder was stored in air tight, high density poly ethylene containers before extraction. Extraction was performed by using soxhlet apparatus (12 hours), carried out first with petroleum ether (60-80ºC) to defat the material. The defatted material was then extracted with methanol to get methanolic extract, then concentrated for further studies at reduced pressure and temperature in a rotary evaporator and tested for presence of secondary metabolites by different phytochemical tests.

Preliminary Phytochemical analysis:

The laf extract was screened for the phytochemical components using the standard method. The phytochemical components analysed were alkaloids, steroids, starch, proteins glycosides, saponins, flavonoids, tannins and cardic glycosides.

Animals

Amimal:	Adult albino mice
Weight:	18-39g
Plant:	*Gymnosporia emerginata*,
Part used:	Leaves
Collected:	S.V University Tirupathi
Period:	Month of June 2010
Authenticated by:	Dr. Madhava Chetty Botanist S.V University
Method of Extraction :	Soxhlet extractor
Percentage of yield:	35g

Phytochemical test

Phytochemical tests on the extract and fractions were performed using standard procedures[6].

Pentylenetetrazole (PTZ)**-induced convulsion test**

Albino mice were randomly divided into three groups (n= 5/group). Group 1 (control) received the vehicle (10 ml/kg, 40% Tween 80 + DMSO (1:1) solution, p.o). Group II received the methanolic extract of *Gymnosporia emerginata*, (300 mg/kg, p.o), while group III received diazepam (2 mg/kg, i.p). Thirty minutes later, pentylenetetrazole (PTZ) (sigma, 60 mg/kg, s.c) was administered to all the

animals. The animals were observed for the time of onset of myoclonic spasms and tonic – clonic phases of seizures. Percentage protection of mice was also recorded in each group. Animals devoid of seizures/convulsion without subsequent death during the 60 minutes observation period were considered protected [7, 8, 9].

Phenobarbitone induced sleeping time

Adult albino mice were randomly divided into three groups (n= 5/group). Control (group 1) animals were treated with the vehicle (10 ml/kg, 40% Tween 80 + DMSO (1:1) solution, p.o). Mice in the group II were treated with the methanolic extract of *Gymnosporia emerginata*, (300 mg/kg, p.o.), while group III received diazepam (Hoffman-la Roche, 2 mg/kg, i.p). These treatments were carried out 30 minutes before the administration of phenobarbitone sodium (Renaudin France, 35 mg/kg, i.p) [10] to all the groups. Each mouse was observed for the onset (latency) of sleep and the duration of sleep using the loss of righting reflexes as the criterion for onset of sleep and the duration of sleep or hypnosis as the time the animal presented a loss of postural reflexes.

Statistical analysis

Data analyzed using One Way Analysis of Variance (ANOVA, SPSS Version 16) and expressed as mean ± SEM and comparisons was done using Dunnet's test as post-hoc. Difference between means were regarded significant at $P<0.05$.

RESULTS

Phytochemical constituents

The phytochemical studies revealed the presence of carbohydrates, alkaloids, glycosides, reducing sugar, resins, flavonoids and terpenoids and the absence of tannins, saponins and acidic compounds.

Pentylenetetrazole-induced convulsion test

The methanolic extract of *Gymnosporia emerginata*, significantly ($P<0.05$) prolonged the onset of both myoclonic spasms (MS) and tonic-clonic phases of seizures (TCS) induced by pentylenetetrazole.

Phenobarbitone induced sleeping time

Results indicated that methanolic extract of *Gymnosporia emerginata* significantly ($P<0.05$) reduced the latency for the onset of sleep and potentiated the duration of sleep at all the doses tested when compared with the control.

Table 1: Effects of extract and fractions on pentylenetetrazole-induced convulsion

Treatment	Dose (mg/kg)	Latency
Control	-	212.0±41.5
Diazepam	2	835.1±12.9**
MEGE	300	421.2±132.8**

n=5, Values are expressed as Mean ± SEM: Significance **P<0.01, *P<0.05 using ANOVA, post hoc Dunnet's test compared to control.

Table 2: Effects of extract and fractions on phenobarbitone-induced sleep time

Treatment	Dose (mg/kg)	Sleep Time (min)	
		Latency	Duration
Control	-	24.30±5.77	184 ± 11.54
MEGE	300	19.67±1.46*	211.06±2.42**
Diazepam	2	18.45 ± 3.46	184 ± 8.66**

n=5, Values are expressed as Mean ± SEM: Significance *P<0.05, **P<0.01, ANOVA, post hoc Dunnet's test compared to control.

DISCUSSION

The results obtained in the study showed that the extract and fraction of *Gymnosporia emerginata*, possesses anticonvulsant and sedative activity. The extract significantly prolonged the onset of both myoclonic spasms as well as tonic–clonic phases of seizure in mice. The effect of pentylenetetrazole induced seizures is an indication of possible effectiveness of the methanolic extract of *Gymnosporia emerginata*, against absence seizures as drugs that inhibit pentylenetetrazole-induced convulsions are generally effective against absence seizures [11, 12]. The reduction in the latency time and prolongation of the duration of sleep is suggesting of the central depressant effects of the extract and fractions. Decrease in latency of onset and prolongation of duration of sleep by the extract and fractions is an indication of central inhibition through the stimulation of the CNS inhibitory pathways. Hence the anticonvulsant and sedative activity of the methanolic extract of *Gymnosporia emerginata* tend to suggest a central inhibitory activity as their possible mechanism of action. Anticonvulsant drugs such as barbiturates and benzodiazepines exhibit their effects through enhancement of gamma amino

butyric acid (GABA) receptors chloride channel complex which is a GABA/ Benzodiazepine mediated inhibition pathway in the central nervous system (CNS) [13]. Pentylenetetrazole induces convulsion by inhibiting the $GABA_A$ receptor-chloride channel complex [14, 15] and therefore agents that abolishs or tend to reduce the effects of pentylenetetrazole possibly acts through the stimulation of such receptors. Benzodiazepines as well as certain anticonvulsants exhibit pharmacological actions through the reduction of sedation and induction of sleep by antagonizing the GABA receptor/ chloride channel complex[16]. The phytochemical analysis showed the presence of flavonoids in methanolic extract of *Gymnosporia emerginata*. The phytochemicals in the methanolic extract of *Gymnosporia emerginata*, was comparable with those present in the methanol extract which has been documented [17]. Flavonoids have been implicated in various pharmacological actions including anticonvulsant and CNS depressant activity[18]. Since flavonoid is the only phytochemical that is found present in the active extract and fractions. This is more so since flavonoids have been accorded central inhibitory and neuromodulatory effects [19]. In conclusion, the results indicated that the leaf of *Gymnosporia emerginata*, exhibited significant anticonvulsant and sedative effects that support the evidence for its folkloric use, while these neuropharmacological effects might possibly be due to the presence of flavonoids. Meanwhile further studies on the purification and structural elucidation of the active phytochemical is ongoing.

REFERENCES

1. Medina JH, Viola H, Wolfman C, et.al., Overview-flavonoids: A new family of benzodiazepine receptor ligands. Neurochemistry. 1997; 22 (4): 419-425.
2. Okoye T.C., Akah P.A. and Omeke C.P. Evaluation of the Anticonvulsant and muscle relaxant effects of the methanol root bark extracts of Annona senegalensis. Asian pacific J. Trop. Med. 2010; 3 (1): 25-28.
3. Gribel G, Perrault G Tan S Shoenaker H, Sanger DJ. Pharmacological studies on synthetic flavonoids: comparision with diazepam. Neuropharmacology. 1999; 38 (7): 965-77.
4. Rocha FF, Lapa AJ, De Lima TCM. Evaluation of the Anxiolytic –like effects of Cecropia glazioui Sneth in mice. Pharmacol Biochem Behav. 2002; 71: 183-190.
5. Yadav IK, Jaiswal D, singh H, Mishra A, Jain DA. Anti-HIV Drugs From Natural Sources. Pharmaceutical research.2009; 1:93-100.
6. Kokate. CK, Practical Pharmacognosy. 4th ed. Delhi: Vallabhprakashan; 1994

7. Okoye TC, Aguwa CN, Akah PA, Nworu CS. Anticonvulsant and sedative effects of leaf extracts of *Stachytarpheta cayennensis.* J. Trop. Med. Plants 2008; 9 (1): 17-22.
8. Dunham NW, Miya TS. A Note on a simple and Mice. J. Am. Pharmaceut. Assoc. Scientific Edit., 1957; XLVI: No. 3.
9. Mukherjee PK. Quality Control of herbal Drugs. New delhi, India: Business Horizons Publishers; 2007: 573-574.
10. Miya TS, Holok HGO, Yim GRW, Spratto GR. Laboratory Guide in Pharmacology, Minneapolis: Burgess publishing Co.; 1973: 44-46.
11. McNamara JO. Drugs Effective in the Therapy of the Epilepsics. In: Gilman AG, Limbird LE, Hardman JG, Editors. Goodmans and Gilmans the Pharmacological Basis of therapeutics 10th edition. Newyork: McGraw-Hill; 2001: 521-547
12. Kasture, V.S., Chopde, C.T., Deshmukh, V.K. Anticonvulsive activity of Albizzia lebbeck, Hibiscus rosasicnensis and Butea monosperma in experimental animals. J. Ethanopharmacol. 2000; 71: 65-75.
13. Corda MG, Giorgi O, Longoni B, Orlandi M, biggio, G. decrease in the function of gamma amino butyric acid coupled chloride channel produced by repeated administration of pentylenetetrazole in rats. J Neuroscience 1990; 55: 1216-1221.
14. Rang HP, Dale MM. Anxiolytic and Hypnotic drugs. In: Rang and Dales Pharmacology. 6th edition. Elsevier: Churchill, Livingstone; 2007: 538.
15. Paladini AC, Marder M, Viola H, Wolfman C eta al. Flavonoids and central nervous system: from forgetten factors to potent anxiolytic compounds. J Pharm Pharmacology. 1999; 51 (5): 519-526.
16. Medina JH, Viola H, Wolfman C, Marder M et al. Overview-flavonoids: A new family of benzodiazepine receptor ligands. Neurochem. 1997; 22 (4): 419-425.
17. Okoye T.C., Akah P.A. and Omeke C.P. Evaluation of the Anticonvulsant and muscle relaxant effects of the methanol root bark extracts of Annona senegalensis. Asian pacific J. Trop. Med. 2010; 3 (1): 25-28.
18. Gribel G, Perrault G Tan S Shoenaker H, Sanger DJ. Pharmacological studies on synthetic flavonoids: comparision with diazepam. Neuropharmacol. 1999; 38 (7): 965-77.
19. Rocha FF, Lapa AJ, De Lima TCM. Evaluation of the Anxiolytic –like effects of Cecropia glazioui Sneth in mice. Pharmacol Biochem Behav. 2002; 71: 183-190.