

Original Research Article

Sedative and Anticonvulsant Activities of the Ethanol Root Extract of *Flemingia chappar* Benth

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Abstract

Purpose: To investigate the sedative, hypnotic and anticonvulsant activities of the ethanol extract of the roots of the *Flemingia chappar* (ERFC) on the central nervous system (CNS) of mice.

Methods: The ethanol extract of the roots of *F. chappar* in doses of 200, 400 and 600 mg/kg, *p.o.*, was studied in mice for its sedative effect by evaluating its locomotor activity; its hypnotic effect was assessed by measuring pentobarbital-induced sleeping time, while anticonvulsant effect was determined by evaluating its activity on maximal electroshock-induced and pentylenetetrazole-induced seizures. The latency of tonic convulsions and number of animals protected from tonic convulsions were noted.

Results: ERFC (200 - 600 mg/kg) significantly ($p < 0.05$) decreased locomotor activity. ERFC also produced dose-dependent prolongation of pentobarbitone sleeping time. In addition, ERFC (400 and 600 mg/kg) significantly ($p < 0.05$) reduced the duration of seizure induced by maximal electroshock (MES). The same dose also protected from pentylenetetrazol-induced tonic seizures and significantly ($p < 0.05$) delayed the onset of tonic seizures.

Conclusion: The results indicate that the ethanol root extract of *F. chappar* has sedative and anticonvulsant activities, thus justifying its use in traditional medicine for epilepsy

Keywords: *Flemingia chappar*, Anticonvulsant activity, Pentylenetetrazole, Electroshock seizure, CNS depressant.

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INTRODUCTION

Epilepsy and seizure are synonymous terms describing chronic neurological dysfunction characterized by recurrent, spontaneous seizures [1]. Seizures can be viewed as resulting from an imbalance between excitatory and inhibitory process in the brain. The proposed mechanism for the generation and spread of seizure activity within the brain include abnormalities in the membrane properties of the neurons, changes in the ionic micro-environment surrounding the neuron, decreased inhibitory

neurotransmission, which is primarily mediated by γ -aminobutyric acid (GABA), or enhanced excitatory neurotransmission which is primarily mediated by the amino acid glutamate [2].

Currently, several antiepileptic drugs are available to treat epilepsy. These antiepileptic drugs often cause many side effects such as chronic toxicity and teratogenicity [3]. Herbal medicines are frequently a source of new therapeutics. Medicinal plants used for the therapy of epilepsy in traditional medicine have been shown to possess promising anticonvulsant

activities in animal models. It is also possible to discover novel lead anti-seizure drugs from these plants that could offer protection against seizures by known or other mechanisms [4].

Flemingia is a genus of flowering plants in the legume family, Fabaceae. It belongs to the sub-family Faboideae (Papilionaceae) and is the major group of angiosperms, native of the tropical and subtropical regions of the world. About 15 species occur in India [5] out of which three are available in Uttarakhand including *Flemingia strobilifera*, *F. chappar* and *F. macrophylla*.

Flemingia chappar (Syn: *Mogahnia chappar*), commonly known as Salpan and Galfulli, belongs to the family Fabaceae. *F. chappar* is a shrub 0.9 - 1.2 m high, the branches are terete, appressedly hairy and pubescent. In India it is distributed in Bengal, Bihar, S. India, Uttarakhand and Gujarat. The leaves are 1-foliolate, cordate and apex is acuminate. The flowers are small, yellowish and pedicel is very short, forming axillary and terminal large bracted racemes, while pods are 8 - 12 mm long, clothed with bright red glands. Traditionally, the roots have been used to treat epilepsy, hysteria, and also as a hypnotic and to relieve pain [6]. The plant has also been used as vermifuge, anticancer, antifungal and anti-filarial [5].

A literature survey revealed that the roots of *F. chappar* contain a chromenochalcone, flemichapparin A; a pterocarpan, flemichapparin B; and a coumestan, flemichapparin C. The roots also contain an anthocyanin, and two sugars (galactose and rhamnose), β -sitosterol, 7-hydroxyflavanon, Flemingin A, B and C, homoflemingin and desoxy-homoflemingin [7]. Traditional healers of Jashpur region of chhaattisgarh use the root in the treatment of epilepsy and insomnia [8]. However, no scientific data are available to validate these folkloric claims. The aim of the present study, therefore, was to evaluate the sedative and anticonvulsant potentials of the ethanol extract of the roots of *F. chappar* in experimental animal models, to determine if there is any justification for the ethnomedical use of the plant root in the management of convulsion and epilepsy in some rural communities of India.

EXPERIMENTAL

Plant material

The roots of *F. chappar* were collected from the local areas of Kaladungi near National Corbett Park (Nainital) in the month of Jan 2008. The plant was identified and authenticated by Dr.

H.D. Pandey, a senior botanist and by GC Joshi, Research Officer-in-Charge, R.R.I (Ayu.) Tarikhet (Ranikhet), Uttarakhand, India. A voucher specimen (no. COP/IFTM-535) has been preserved in the herbarium of the Department of Pharmacognosy, College of Pharmacy, IFTM, Moradabad for future reference.

Preparation of extract

F. chappar roots were washed, dried and coarsely powdered in a mechanical grinder; it was then extracted in a Soxhlet extractor using 95 % ethanol as solvent. The extract was dried in a rotary vacuum evaporator and kept in desiccator until further use.

Experimental animals

Swiss albino mice of either sex, weighing between 12 - 35 g, were used in this study. The animals were housed in polypropylene cages maintained at standard conditions of 12 h light /12 h dark cycle; 25 ± 3 °C, 45 - 55 % relative humidity) and had free access to standard rat feed and water *ad libitum*. All the animals were acclimatized to laboratory conditions for a week prior to commencement of experiments. Animal care and research protocols were based on international guidelines by National Institute of Health (NIH) [9]. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision on Experiments on Animals, New Delhi, India (no. 837/ac/04/CPCSEA), *vide*, reg no. 1/837/ac/PH/10.

Drugs and chemicals

Pentylentetrazole (PTZ, Sigma Aldrich, USA), pentobarbitone (Sigma Aldrich, USA), diazepam (Cipla, Ahmedabad, India), and phenytoin (Sigma Aldrich, USA) were the major chemicals and drugs used.

Phytochemical screening

The freshly prepared extract of the roots of ERFC was subjected to phytochemical screening tests for the detection of various constituents [10].

Determination of total phenolics

The total phenol content of the plant extract was determined by Folin-Ciocalteu method [11]. The extract solution (10 %, 100 μ l) was mixed with 2 ml of Folin-Ciocalteu reagent and 1.6 ml of

sodium carbonate, shaken well and kept for 2 h. The absorbance was measured at 750 nm using a UV spectrophotometer (Shimadzu 1700). Using gallic acid monohydrate as standard, a standard curve was prepared and linearity was obtained in the range of 2.5 – 25.0 µg/ml, and total phenol content was expressed as gallic acid equivalent in %w/w of the extract. The experiment was carried out in triplicate.

Determination of total flavonoids

The total flavonoid content of the extract was determined by a colorimetric method [12]. The extract (10 %, 0.5 ml) was mixed with 1.5 ml methanol, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a UV spectrophotometer (Shimadzu 1800). Using quercetin as standard, a standard curve was prepared and linearity was obtained in the range of 1 - 10 µg/ml, and used to compute total flavonoid content which was expressed as quercetin equivalent in %w/w of the extract. The experiment was carried out in triplicate.

Acute oral toxicity study

Acute oral toxicity studies were performed as per internationally accepted protocol (OECD guidelines 423) in albino mice [13]. The ethanol extract was given at dose levels of 5, 50, 300 and 2000 mg/kg body weight. The animals were observed individually after dosing during the first 24 h, especially during the first 4 h and daily thereafter for a total of 14 days.

Test for locomotor activity

The spontaneous locomotor activity of each mouse was recorded for individual mice for 10 min using an actophotometer. (INCO, Ambala, India) Three doses of the extract (200, 400 and 600 mg/kg *p.o.*) were administered 60 min prior to the test while diazepam (4 mg/kg *i.p.*), used as a reference standard, was given 30 min prior to the test. The control group was treated with 2 %w/v Tween 80 orally 60 min prior to the test [14].

Pentobarbital-induced sleep test

The vehicle (2 %w/v Tween 80) and ERFC (200, 400 and 600 mg/kg *p.o.*) were administered 25 min. before *i.v.* administration of pentobarbital sodium. Sleep time was considered as the difference between the time of loss and recovery of righting reflex [15].

Anticonvulsant activity

Electrically-induced seizure model

In the electrically-induced seizure experiment, the maximal electroshock (MES) method was employed [13]. Seizure was induced in the animals by electroconvulsive shock (50 mA for 0.2 s) via a corneal electrode using an electroconvulsometer. The various phases of convulsion produced were flexion, extension, clonus and stupor. The duration of hind limb tonic extension (HLTE) was compared with control. Decrease in the duration of hind limb extension was considered a protective action. The animals were divided into 5 groups of 6 animals each. Group I served as control (vehicle) group (treated Tween 80, 0.25 ml, *p.o.*, 60 min). Groups II, III and IV served as test groups and were treated with the extract (200, 400 and 600 mg/kg *p.o.* for 7 days), respectively, while group V was the reference group and was treated with phenytoin (20 mg/kg *i.p.*, 30 min) prior to the induction of convulsion.

Chemically-induced seizure model

Seizure was induced in the mice by administration of PTZ (80mg/kg) intraperitoneally. Jerky movement, convulsant and protection (%) were recorded. The animals were divided into 5 groups of 6 animals each and they were treated as described for electrically-induced seizure tests.

Statistical analysis

The results were statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test [16]. The results were expressed as mean ± standard error mean (SEM). *P* values of < 0.05 and < 0.01 were considered as significant, compared to control. All computations were performed using Graph Pad Prism V. 4 (GraphPad, Inc. USA).

RESULTS

Phytochemical screening

Preliminary phytochemical investigation of the extract (ERFC) revealed the presence of carbohydrates, tannins, phenolic compounds, steroids and flavonoids.

Total phenolics and total flavonoids

The results obtained showed the presence of 11.25 %w/w phenolic (calculated as gallic acid) and 3.15 %w/w of flavonoids in the extract.

Acute toxicity

In the acute oral toxicity study there was neither mortality nor any toxicity symptoms up to 2000 mg/kg in albino mice for up to 72 h after oral administration of the extract, and hence > 2000 mg/kg was taken as LD₅₀ cutoff value and 1/10th of this dose, i.e., 200 mg/kg, was selected for further studies.

Locomotor activity

ERFC at all doses (200, 400 and 600 mg/kg p.o.) and diazepam (4 mg/kg i.p) showed significant ($p < 0.05$) reduction in the locomotor activity (Table 1).

Table 1: Effect of oral ERFC and i.p. diazepam on locomotor activity

Group	Actophotometer score in 10 min
Control	319.43±9.45
ERFC (200 mg/kg)	255.48±7.38*
ERFC (400 mg/kg)	222.82±12.08*
ERFC (600 mg/kg)	180.42±14.23*
Diazepam (4 mg/kg)	120.16±11.14*

Values are expressed as mean ± S.E.M, (n = 6); *significant at $p < 0.05$ compared with control

Pentobarbitone-induced sleeping time

ERFC (400 and 600 mg/kg p.o) significantly ($p < 0.05$) increased pentobarbitone-induced sleeping time. No significant effect was observed with 200 mg/kg dose of the extract (Table 2).

Table 2: Effect of *F. chappar* (ERFC) ethanol root extract on pentobarbitone- induced sleeping time

Treatment	Onset of action (min)	Duration of Action (min)
Control	5.85±0.47	128.53±0.16
ERFC (200mg/kg)	6.12±0.52	140.48±0.28
ERFC (400mg/kg)	5.91±0.18	150.36±0.43*
ERFC (600mg/kg)	5.65±0.27	208.25±0.84*

Pentobarbitone (40 mg/kg, i.p) 30 min post treatment of the vehicle and drugs. Values are expressed in mean ±S.E.M, n=6 *significant at $p < 0.05$ compared with control Dunnet's test

Anticonvulsant activity

Maximal electroshock produced hind limb tonic extension seizures (HLTE) in all the animals groups. The vehicle-treated mice showed tonic hind limb extension duration of 13.75 ± 0.51 s. ERFC (200 mg/kg) significantly ($p < 0.05$) reduced the latency, but did not alter the incidence of seizures elicited by maximal-electroshock to any significant extent while doses

of 400 and 600 mg/kg protected 50 and 62.5 % of the mice, respectively, and also significantly ($p < 0.05$) reduced the duration of seizures. However, phenytoin completely inhibited maximal electroshock (MES-induced) tonic seizures in all the animals tested (Table 3).

Pentylenetetrazole produced tonic seizures in all the animals tested. An extract dose of 200 mg/kg protected 25 % of the animals against seizures and did not affect the onset of seizure to any significant extent. Higher extract doses (400 and 600 mg/kg) provided 62.5 and 100.0 % protection against seizures, and significantly ($p < 0.05$) delayed the latency of seizures. The standard antiepileptic drug, diazepam, completely protected the animals against seizures (Table 4).

DISCUSSION

The ethanol extract of the roots of *F. chappar* (ERFC) potentiated the sleep induced by pentobarbitone suggesting that it possesses some sleep-inducing property. Spontaneous motor activity data showed that the extract dose of 600 mg/kg decreased the frequency and amplitude of motor movements. This reduction could be attributed to the sedative effect of the extract.

The most widely used animal seizure models are the traditional MES and PTZ test [16]. Prevention of seizure induced by PTZ in laboratory animals is the most commonly used preliminary screening test for characterizing potential anticonvulsant drugs [16]. MES test is considered to characterize therapeutic efficacy against generalized tonic-clonic seizures. By contrast, PTZ test represents a valid model for human generalized myoclonic seizure and absence of seizure [16]. Extract doses of 400 and 600 mg/kg produced lower mortality but increased time of onset of clonus compared to control. Epileptic agents such as PTZ exert their epileptic activity by inhibiting gamma aminobutyric acid (GABA) activity and increasing electrical activity in brain.

Diazepam and phenobarbitone are antiepileptic drugs, which usually produce their effect by enhancing GABA-mediated inhibition in the brain, thus reducing electrical activity in the brain cortex [17]. It is, therefore, possible that the antiepileptic effect used in this study might be due to the activation of GABA. Most of the flavanoids interact with GABA receptors in brain and modulate its function, whereas isoflavonoids have protective effect against PTZ-induced

Table 3: Effect of ethanol extract of the roots of *F. chappar* (ERFC) on maximal electroshock (MES)-induced seizures in mice

Treatment (mg/kg)	Duration of seizure phase (s, mean±SEM)				
	Flexion	Extension (HLTE)	Clonus	Stupor	Protection (%)
Control	4.22±0.12	13.75±0.51	15.98 ± 0.23	95.54 ± 0.45	0
Phenytoin, 20	1.88±0.19	0.00*	7.36 ± 0.20	42.93 ± 0.77	100
Extract 200	4.20±0.20	11.54±0.32	11.08 ± 0.33	71.98 ± 0.31	33.3
400	2.63±0.22	5.70±0.22*	9.57 ± 0.31	63.46 ± 0.37	50
600	2.10±0.16	2.46±0.46*	8.28 ± 0.23	51.43 ± 0.34	66.66

Values are expressed as mean ± SEM (n = 6); *significant at p < 0.05 compared with control

Table 4: Effect of ethanol extract of the roots of *F. chappar* (ERFC) on pentylenetetrazole (PTZ)-induced seizure in mice

Treatment (mg/)	Mortality, seizure type and onset (s, mean±SEM)				
	Myoclonic jerk	Clonus	Extensor	Mortality	Protection (%)
Control	45.63±0.21	91.70±0.34	284.45±0.3	370.12±0.42	0
Diazepam, 4	280.15±0.51*	815.50±0.34	Nil	nil	100
Extract 200	135.85±0.30*	450.12±0.46	650.16±0.45	730.48±0.54	33.3
400	185.05±0.41*	540.25±0.48	755.45±0.43	820.89±0.77	62.5
600	200.05±0.81*	695.67±0.57	820.73±0.87	NIL	83.3

Values are expressed as mean ± SEM (n = 6); *significant at p < 0.05 compared with control

seizures. Hence the extract may antagonize seizure elicited by PTZ in mice by affecting gabaergic mechanism since the extract contains flavonoids. [18].

MES-induced tonic seizures can be prevented either by drugs that inhibit voltage-dependent Na⁺ channels, such as phenytoin and valproate, or by drugs that block glutaminergic excitation mediated by n-methyl-D-aspartate (NMDA) receptors such as felbmate [19]. The extract probably follows any one of the above mechanisms. The extract, at a dose of 200 mg/kg exerted little effect on the total duration of hind lib tonic extension (HLTE) but at doses of 400 and 600 mg/kg, respectively, the reduction in HLTE was pronounced. The reference drug used, phenytoin abolished tonic extension phase.

Although it would be helpful to isolate bioactive principles of the extract responsible for the observed activities of the plant material, our findings justify the traditional use of this plant in the control and/or treatment of insomnia and epilepsy.

CONCLUSION

The ethanol extract of *F. chappar* root possesses sedative and antiepileptic activity. The anticonvulsant effects of the ethanolic root extract may be via mechanisms that have not yet been elucidated. Therefore, further studies are required to determine the precise mechanism(s)

of the extract as well as the safety profile of the plant.

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