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 *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.

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
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-
hydroxyflavanon, Flemingin A, B and C,
homoflemingin and desoxy-homoflemingin [7].
Traditional healers of Jashpur region of
chhattisgarh 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
cosely powdered in a mechanical grinder; it was
then extracted in a Soxhlet extractor using 95 %
ethanolo 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 polypylene 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

Pentylenetetrazole (PTZ, Sigma Aldrich, USA),
pentobarbitone (Sigma Aldrich, USA), diazepam
(Cipla, Ahemdabad, 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 μl) 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 p.o. 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 50 % 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 phenobarbital 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 flavonoids interact with GABA receptors in brain and modulate its function , whereas isoflavonoids have protective effect against PTZ-induced
IOactive and valproate, act as well as the safety profile of the 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.

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.

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

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

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Duration of seizure phase (s, mean±SEM)</th>
<th>Flexion</th>
<th>Extension (HLTE)</th>
<th>Clonus</th>
<th>Stupor</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.22±0.12</td>
<td>15.98 ± 0.23</td>
<td>95.54 ± 0.45</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenytoin, 20</td>
<td>1.88±0.19</td>
<td>7.36 ± 0.20</td>
<td>42.93 ± 0.77</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extract 200</td>
<td>4.20±0.20</td>
<td>11.08 ± 0.33</td>
<td>71.98 ± 0.31</td>
<td>33.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.63±0.22</td>
<td>5.97 ± 0.31</td>
<td>63.46 ± 0.37</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.10±0.16</td>
<td>8.28 ± 0.23</td>
<td>51.43 ± 0.34</td>
<td>66.66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

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

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