

### ANXIOLYTIC AND ANTIEPILEPTIC ACTIVITY OF AERIAL PARTS OF *FLEMINGIA MACROPHYLLA* IN RODENTS

CH. BHARGAVI\*, B. VENKATARAMANA

*For Author affiliations see end of the text*

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#### ABSTRACT

The present study was undertaken to evaluate the anxiolytic and anti epileptic activity of ethonolic extract of *Flemingia macrophylla* by using different extract concentrations in rodents. Anxiolytic activity of FME was estimated by using Elevated plus maze test and light-dark model along with Mono Amino Oxidase enzyme levels in mice and the anticonvulsant activity of FME was estimated in rats by using MES , PTZ methods and those showing response were divided into four groups of six animals each. Group-I (Normal), Group-II (Diazepam 2mg/ Kg , p.o) ,

Group-III (FME 20mg/Kg in mice, 100mg/Kg in rats, p.o), Group-IV(FME 50mg/Kg in mice, 200mg/Kg , p.o). Animals treated with FME had shown a significant increase in enzyme levels and significant decrease in anxiety and onset of convulsions when compared with the diazepam treated animals. The ethanolic extract of *Flemingia macrophylla* afforded significant protection against anxiety and epilepsy. The activity may be due to the presense of flavonoids& glycosides.

**KEY WORDS:** Anti anxiety, Anti epileptic, *Flemingia macrophylla* , Diazepam, Pentylene tetrazole.

#### INTRODUCTION

Anxiety is a universal feeling state and is part of the fabric of everyday life. Feelings of anxiety and fear often are unpleasant emotions commonly caused by the perception of actual or potential (anticipatory) danger that threatens the security of the individual<sup>[1]</sup>. Epilepsy is a physical condition that occurs when there is a sudden, brief change in how the brain works. When brain cells are not working properly, a person's consciousness, movement, or actions may be altered for a short time. These physical changes are called epileptic seizures. Epilepsy is therefore sometimes called a seizure disorder<sup>[1]</sup>.

A major revolution in clinical neuropharmacology and psychopharmacology was herald during the past years. The natural compounds have played a major role in neurological disorders. A number of herbal drugs traditionally employed in the Indian system of medicine Ayurveda. Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care<sup>[2]</sup>.

*Flemingia macrophylla* belongs to the family Fabaceae. In some areas, such as Ghana, *Flemingia macrophylla* remains green throughout the year and retains most of its leaf during the dry season, making it suitable as a dry-season browse species. In Indonesia and Malaysia, the leaves are used medicinally. In China, a decoction is used to bathe sores and swellings, while in Taiwan it is an antipyretic for treating postpartum fever and is used to treat paralysis and pain in the joints. Some the plants of this were proved as antibacterial agent, anthelmintic agent. As a folk medicine it is used in ulcers, vertigo, vitiligo, hysteria, vermifuge. Roots are used for the external application for ulcers & swellings. Various plant parts used in spleen complaints, smallpox, prolapsusani, cholera, dysentery and in blindness. The main aim of this study is to estimate the antianxiety & anticonvulsant activity of aerial parts of the plant based On the study of neuroprotective activity of flavanoids from the aerialparts of *Flemingia macrophylla*.

### MATERIALS & METHODS

#### Plant material

*Flemingia macrophylla* aerial parts were collected from the Talakona forest in Tirupathi, Andhrapradesh, India and authenticated by the normal pharmacognostic procedures by Dr. K. Madhavchetty, a senior botanist in Sri venkateswara University(SVU), Tirupathi, India.

#### Preparation Of Extraction

The plant parts were dried under room temperature (1000g) were defatted with petroleum ether and extracted with 95% ethanol by continuous hot percolation method using Soxhlet apparatus. The extract was made free from the solvent by evaporation. The extract was tested for phytochemical screening.

#### Drugs and chemicals

Diazepam (Ranbaxy Laboratories Ltd, India), Pentylene tetrazole (Sigma-Aldrich, St.Louis, MO63103, USA) were used for the study. All the solvents used for the extraction process are of Laboratory grade and they are purchased from local firms.

#### Animals

Swiss albino mice of either sex (25-30 g) were maintained for 7 days in the animal house under standard conditions, temperature ( $24 \pm 10^\circ\text{C}$ ), relative humidity (45-55%) and 12:12 light: dark cycle. The animals were fed with standard rat pellet and water ad libitum. The animals were allowed to acclimatize to laboratory conditions 48hr before the start of the experiment.

#### Light-Dark Model<sup>[9]</sup>

Light dark box is a rectangular box of 46 x 27 x 30 cm (l x b x h), which is divided into 2 compartments 1/3 rd is for the dark compartment and 2/3 rd served as light compartment. Extract/vehicle or standard drugs will be administered through per oral route. Sixty min after oral administration the mouse were placed individually on the light compartment and observe for a period of 5 min. Number of locomotion, time spent in light and dark zones and number entries in light, dark zone were observed during this observation period.

#### Experimental model

The animals were divided into 4 groups (n=6), Group-I (Normal) treated with saline (0.9%), Group-II (Standard) treated with the Diazepam (2mg/Kg), Group-III, IV (test animals) treated with extract (20mg/Kg, 50 mg/Kg). The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of the SRM University, Kattankulathur, Tamil Nadu, India. All the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA)

#### Pharmacological activities

##### Elevated Plus Maze<sup>[8]</sup>

The Elevated plus-maze test is described elsewhere. The apparatus comprises of two open arms (35 cm x 5 cm) and two closed arms (30 cm x 5 cm x 15cm) that extend from a common central platform (5 cm x 5 cm). The floor and walls of the closed is painted black. The entire maze is elevated to height of 50 cm above the floor level. Mice of 18-22g weight were housed in pair for 10 days prior to testing in the apparatus. During this time the mice are handled by the investigator on alternate days to reduce stress. A group consisting of 6 mice was used for each dose level. One hour after oral administration of the vehicle or standard or extract, each mouse was placed in the center of the maze facing towards one of the open arm. During a five minutes session, the following parameters were noted; Number of entries into open arm, Number of entries into closed arm, Time spent in the open arm, and Time spent in the closed arm.

##### Mono Amine Oxidase Assay (MAO)<sup>[10]</sup>

Mouse brain mitochondrial fraction will be prepared by cutting the brain sample into small pieces and rinsed in 0.25m sucrose 0.1 m tris 0.02m EDTA (pH7.4) to remove blood. The pieces will be homogenized for 45 sec in a potter-elvehjem homogenizer with 400 ml of the same medium. The homogenate will be centrifuged at 800xg for 10min and the pellets will be discarded. The supernatant will be centrifuged at 12,000xg for 20 min in the same medium. The precipitate will be washed twice more with 100ml of sucrose tris EDTA and resuspended in 50ml of

the medium. The protein concentration will be adjusted to 1 mg/ml. MAO activity was assessed spectrometrically.

The assay mixture contain 4mm of serotonin as specific substrates for MAO-A, 250µl solution of the mitochondrial fraction and 100mm sodium phosphate buffer (pH7.4) up to the final volume of 1ml, the reaction will be allowed to proceed at 37 ° c for 20 minutes and stopped by adding 1M HCl (200µl), the reaction product will be extracted with 5ml of butyl acetate, the organic phase was measured at wavelength of 280 nm in a spectrometer. Blank samples will be prepared by adding 1m HCl (200µl) prior to the reaction and worked subsequently in the same manner.

### Assesment Of Antiepileptic activity

#### Pentylentetrazole induced convulsion<sup>[11]</sup>

Male swiss albino mice weighing between 18-25g were divided into four groups, each group comprising of six animals, One hour after vehicle or standard or extract

treatment, PTZ (pentylentetrazole 80 mg/kg) was administered by intraperitoneally(i.p) to all the animals. Each animal was placed in to individual plastic cage and observed initially for 30min and later up to 24 hrs. The following parameters were recorded during test session of initial 30min and up to 24 hrs; Onset of clonic convulsions, Status of animal after 24 hrs.

#### Maximal electroshock test (MES)<sup>[11]</sup>

An alternating current stimulus of 50 Hz and 150 mA through bicornal electrodes will be delivered for 0.2 s to the experimental animals. A drop of 0.9% saline solution should be poured into each eye prior to placing the electrodes. Duration of clonic convulsion and percentage of mortality will be recorded.

### RESULTS

#### Phytochemical Screening

It was found that the alcoholic extract of *Flemingia macrophylla* contains flavonoids, glycosides.

### Assesment of antianxiety activity

Table1: Elevated plusmaze test

Group	Drug treated	Number of entries/5min		Timespent/5min		Time spent in neutral region(counts/min)
		Open arm	Closed arm	Open arm	Closedarm	
I	Normal	6±0.04**	10.24±0.4**	44.2±3.17	191.31±1.3*	53±3.3**
II	Diazepam (2mg/Kg)	10.67±0.3	57.36±0.42	26.91±2.03	245.37±8.67	19±2.4
III	FME (20mg/Kg)	9.3±0.24**	10.01±0.03**	28.51±3.81*	195.77±1.06*	23.7±0.6*
IV	FME (50mg/Kg)	11.6±0.46*	9.11±0.42*	29±1.8**	176.3±0.6**	25.11±1.12**

\*\*P<0.01. \*P<0.05 (Dunnet test, which compares test groups and standard drug group with control group);number of animals per group=6

**Table 2: Light-Dark Model**

Group	Drug treated	Number of entries in light(sec)	Timespent in lightbox(sec)	Total locomotion(counts/min)
I	Normal	7.3±0.47**	20.4±0.9**	140.5±1.9**
II	Diazepam (2mg/Kg)	14.6±0.8	102.2±1.45	90.7±0.9
III	FME (20mg/Kg)	10.6±0.7**	107.7±0.6*	112.6±2.6**
IV	FME (50mg/Kg)	12.1 ±0.6*	100±0.43*	100.48±1.5**

\*\*P<0.01.\*P<0.05 (Dunnet test, which compares test groups and standard drug group with control group);number of animals per group=6

**Table3: Effect of FME on MAO levels**

Drug treated	MAO levels
Control	19.12±0.91**
Diazepam(2mg/Kg)	23.69±1.78
FME(20mg/kg)	24.21± 0.9**
FME(50mg/Kg)	25.02±1.1

\*\*P<0.01.\*P<0.05 (Dunnet test, which compares test groups and standard drug group with control group);number of animals per group=6

### Assesment of antiepileptic activity

**Table4 : Effect Of FME in MES induced convulsions**

Group	Drug treated	Time duration(sec)				Recovery/Death
		Flexion	Extension	Convulsion	Stupor	
I	Normal	53±1.27**	70±2.90*	90.3±0.73**	101.11±3.41**	Recovery
II	Diazepam (2mg/Kg)	24.6±0.18	23±1.10	19.1±0.09	9.9±0.18	Recovery
III	FME (20mg/Kg)	28.3±0.19**	19.21±1.6**	10.2±0.76**	5.5±0.19**	Recovery
IV	FME (50mg/Kg)	27.3±0.2*	17.4±1.2*	9.1±0.29**	3.4±0.2**	Recovery

\*\*P<0.01.\*P<0.05 (Dunnet test, which compares test groups and standard drug group with control group);number of animals per group=6

**Table5: Effect Of FME on PTZ induced seizures**

Group	Drug treated	Onset of clonic convulsions(sec)
I	Normal	80±2.5**
II	Diazepam(2mg/Kg)	37.1±0.31
III	FME(20mg/Kg)	30.33±0.29**
IV	FME(50mg/Kg)	28.1±0.71**

\*\*P<0.01.\*P<0.05 (Dunnet test, which compares test groups and standard drug group with control group);number of animals per group=6

### DISCUSSION

FME treated animals and Diazepam treated animals had shown significant activity in elevated plus maze test particularly in number of entries, time spent **Table:1**. The FMEextract treated animals had shown significant activity than standard (Diazepam) treated animals. In light and dark model also animals had shown more significant activity like standard animals by preferring the light region than dark region **Table:2** and significant increase in enzyme levels**Table:3** Evaluation of antiepileptic activity revealed

that there was a significant activity was shown by the animals than the standard. In MES induced convulsion model there was a significant decrease in extension, convulsion and stupor stage when compared with the Diazepam treated animals **Table:4**. In PTZ the onset of convulsion was decreased in FME treated animals better than the Diazepam treated animals which were recovered at last **Table:5**.

### CONCLUSION

By this study we can conclusively state that *Flemingia macrophylla* has definite beneficial role as an anxiolytic & anticonvulsant. It can serve as a good adjuvant in the present armamentarium of anticonvulsant drugs and support its traditional usage in the treatment of both in anxiety and convulsions. Further studies is required for the detailed studies in isolation of the compounds and pharmacological investigations of the other plant constituents, which have many pharmacological activity reported traditionally and its exact mechanism of action.

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### **AUTHORS AFFILIATIONS AND ADDRESS FOR CORRESPONDENCE**

Ch.Bhargavi,

SRM College of pharmacy, SRM University,

Kattankulatur, Chennai.

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