OPEM
www.opem.org

Oriental Pharmacy and Experimental Medicine 2006 **6(1)**, 21-26

KHUP

Central nervous system stimulating activity of the ethanolic extract of *Fleurya interrupta* Guad. (Urticaceae)

Jamil Ahmad Shilpi[1], Razina Rouf[1], MM Ferdous[2] and Shaikh Jamal Uddin[1,*]

[1]Phytochemical and Pharmacological Research Laboratory, Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh; [2]Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh

SUMMARY

The ethanolic extract of *Fleurya interrupta* Gaud. (Urticaceae) was tested for its possible neuropharmacological effects on experimental animals. For the primary neuropharmacological screening of this plant, the ethanolic extract of its aerial parts was subjected to preliminary evaluation for acute toxicity, antinociceptive activity and central nervous system (CNS) activities. At the doses of 125 and 250 mg/kg, the extract significantly ($P < 0.01$ and $P < 0.001$) and dose-dependently increased the frequency of acetic acid induced writhing in mice. In the pentobarbitone induced sleeping time test, the extract at the above dose levels, significantly and dose-dependently decreased the pentobarbitone induced sleeping time ($P < 0.001$) and increased the time for onset of sleep ($P < 0.001$) in mice. In the open field and hole cross tests, test animals showed an increase in their movement in the both tests from the 2nd observation period (30 min) and persisted throughout the entire experimental period (240 min). These results of the extract may attribute a stimulating action on the CNS. On the basis of these findings, it can be assumed that the extract exerts its stimulating effect on the CNS in mice by interfering with the cortical function or increasing the effect of some CNS stimulating neurotransmitters.

Key words: *Fleurya interrupta*; Acute toxicity; Antinociceptive activity; Central nervous system

INTRODUCTION

Fleurya interrupta (*F. interrupta*) Gaud. (Urticaceae) (Syn. *Loportea interrupta*); locally known as 'Chosra', a annual herb with stinging hairs, leaves alternative, flowers monoecious or dioecious, fruit is blique, and seeds are very scanty albumen, grows throughout Bangladesh (Prain, 1981). It also grows in Fiji, almost Africa to Japan, China, and eastward to queensland and pacific as far as Hawaii's (Smith, 1981). In Hawaii it occurs as common garden weed (Wagner *et al.*, 1999). Locally the plant hair used as stimulant and in dermatitis, leaves past is applied to carbuncle and root extract is diuretic. The air-dried aerial parts of *F. interrupta* contained 11 amino acids, the major one being proline (Dinda *et al.*, 1988) and a relative percentage of the component of fatty acids as calculated form the recorded peak areas of their Me-esters and others unidentified. The presence of unsaturated fatty acids was also confirmed by catalytic hydrogenation of a part of Me-ester mixed with 10% Pd-C followed by gas-liq. chromatographic anal. of the hydrogenated product was found from the plant (Dinda *et al.*, 1990). In search for plants having effect on central nervous system (CNS) and other pharmacological effects we

*Correspondence: Shaikh Jamal Uddin, Phytochemical and Pharmacological Research Laboratory, Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh. E-mail: uddinsj@yahoo.com

are screening commonly available Bangladeshi medicinal plants (Shilpi *et al.*, 2004, 2005; Uddin *et al.*, 2004, 2005), and as a part of the continuation of this research based on the evidence of the use of *F. interrupta* as a stimulating herbs by the folk people, we now report on the effect of the extract of *F. interrupta* on CNS in mice using established neuropharmacological experimental models.

MATERIALS AND METHODS

Plant material and extraction

The plants of *F. interrupta* were collected from Khulna in the month of October 2003 and was identified by the experts of Bangladesh National Herbarium, Dhaka, Bangladesh, where a voucher specimen was deposited (Accession no. DACB-30542). The dried plants, except root, were pulverized into coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The dried and powdered plant parts of *F. interrupta* (500 mg) were subjected to maceration by 95% EtOH (650 ml) at room temperature for 2 days. The resulting extract was filtered and solvent was evaporated using rotary evaporator and yielded approximately 3% w/w. Phytochemical investigation indicated the presence of reducing sugars, tannins, flavonoids and saponins (Harborne, 1984).

Animals

Swiss albino mice of either sex (20 - 25 g) were obtained from the Animal house, Pharmacy Discipline, Khulna University, Khulna. The animals were housed under standard laboratory conditions (relative humidity 55 - 65%, room temperature 23.0 ± 2.0°C and 12 h light : dark cycle). The animals were fed with standard diet and water *ad libitum*. The control vehicle and test substances were administered to the test animals at the dose of 10 ml/kg body weight using a feeding needle.

Acute toxicity test

Test animals were divided into six groups containing six animals in each. Five groups received 62.5, 125, 250, 500, 1,000, 2,000 and 4,000 mg/kg body weight of the ethanol extract of *F. interrupta* orally whereas the control group received normal saline. The general sign and symptoms of toxicity and mortality were recorded for 24 h (Lorke, 1983).

Antinociceptive activity study using acetic acid induced writhing assay

The method of Koster *et al.* (1959) was adopted with minor modification. The animals were orally fed with the extracts (vehicles for control group) at the specified doses (125 and 250 mg/kg body weight). Thirty minutes after administration of the extract and the vehicle, each animal was given 0.7% (v/v) solution of acetic acid (0.1 ml/10 g body weight) interperitoneally (i.p.) to induce abdominal contractions or writhing. Five minutes after the administration of acetic acid, the number of writhing for each animal was counted for 15 min. The number of writhings in the control was taken as 100% and percent inhibition was calculated as follows:

% Inhibition of writhing = 100 – (treated mean/ control mean) × 100

For comparison, the same experiment was carried out with a positive control group treated orally with aspirin (Square Pharmaceuticals Ltd., Bangladesh) at the dose of 50 mg/kg body weights.

Pentobarbitone-induced sleeping time test

The animals were randomly divided into four groups consisting of five mice each. The test groups received *F. interrupta* extract at the doses of 125 and 250 mg/kg body weight while positive control was treated with diazepam (1 mg/kg i.p.) and control with vehicle (1% Tween 80 in water). Thirty minutes later, pentobarbitone (50 mg/kg i.p.) was administered to each mouse to induce sleep. The animals were observed for the latent period (time between pentobarbitone administration to onset of sleep) and duration of sleep (time between

the loss of righting reflex to recovery of righting reflex) (Williamson *et al.*, 1996; Shilpi *et al.*, 2004).

Open field test

This experiment was carried out as described by Gupta *et al.* (1971). The animals were divided into control and test groups containing five mice in each. The test groups received *F. interrupta* extract at the doses of 125 and 250 mg/kg body weight orally whereas control group received vehicle (1% Tween 80 in water). The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min. on 0, 30, 60, 90, 120, 180 and 240 min during the study period.

Hole cross test

The method was adopted as described by Takagi *et al.* (1971). A steel partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The number of passages of a mouse through the hole from one chamber to other was counted for a period of 3 min on 0, 30, 60, 90, 120, 180 and 240 min during the study period.

RESULTS

Acetic acid induced writhing in mice

The crude extract of *F. interrupta*, given orally at 125 and 250 mg/kg body weight significantly ($P < 0.01$ and $P < 0.001$) and dose-dependently increased the frequency of acetic acid induced abdominal constrictions in mice (Table 1). Writhing observed at the above dose levels were 117.07 and 132.90% respectively. Where the writhing inhibition in mice of salicylic acid, commonly used NSAID drug, treated as positive control, was found to be 77.24% (i.e. 22.76% writhing).

Pentobarbitone induced sleeping time in mice

In the pentobarbitone induced sleeping time, the extract significantly ($P < 0.001$) and dose-dependently reduced the sleeping time in mice at the doses of 125 and 250 mg/kg body wt. The extracts also increased the time required for the onset of sleep at the both doses ($P < 0.001$) (Table 2).

Table 1. Effect of *F. interrupta* extract on acetic acid induced writhing in mice

Treatment	Dose[a] (mg/kg, p.o.)	Writhings[b]	% Writhing
Control (1% Tween 80, 10 ml/kg, p.o.)	-	32.8 ± 1.29	100
Aspirin	50	6.6 ± 0.57**	20.12
F. interrupta	125	38.4 ± 0.57*	117.07
	250	43.6 ± 1.60**	132.92

[a]Administered 45 min before 0.7% acetic acid administration (10 ml/kg, i.p.).
[b]Counted for 15 min, starting 5 min after acetic acid administration; values are mean ± S.E. **$P < 0.001$, *$P < 0.01$ vs. control, Student's *t*-test; n = 5.

Table 2. Effect of *F. interrupta* on pentobarbitone induced sleeping time in mice[a]

Treatment	Dose (mg/kg)	Route of Administration	Latent Period (min)	Duration of Sleep (min)
Control (1% tween 80 in water)	10 ml/kg	p.o.	5.72 ± 0.41	65.72 ± 2.55
Diazepam	1	i.p.	4.06 ± 0.27*	83.34 ± 2.33**
F. interrupta	250	p.o.	36.88 ± 2.08**	48.58 ± 2.40**
	500	p.o.	48.78 ± 2.70**	33.16 ± 1.44**

[a]Values are mean ± SEM. **$P < 0.001$, *$P < 0.01$, vs control, students t-test, n = 5.

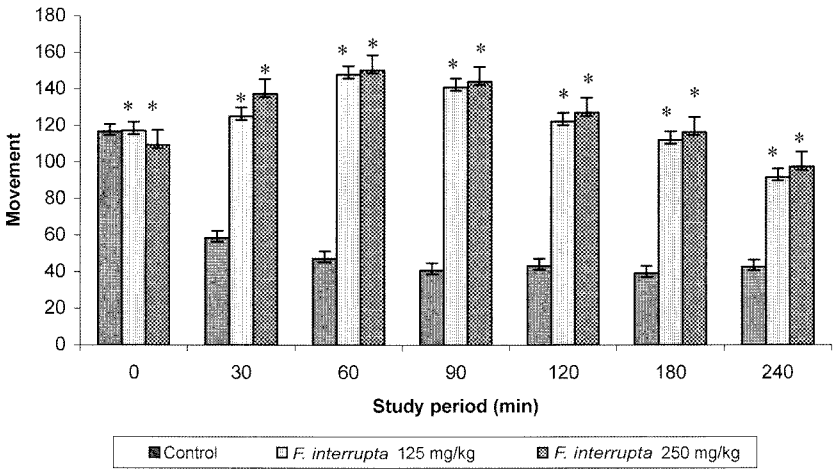

Fig. 1. Effect of the ethanol extract of *F. interrupta* on Open field test. $^{*}P < 0.001$ vs control, students *t*-test, values are mean ± SEM. n = 5.

Open field tests

In the open field test, the extract showed a noticeable increase in locomotion in the test animals from the second observation period at the both doses (125 and 250 mg/kg body weight). But the stimulating action was slowly decreased with the passage of time. The effect was dose dependent and the result was statistically significant ($P < 0.001$) (Fig. 1).

Hole cross test

In the hole cross test, the stimulating action also observed of the extract from the second observation time period in the test animals at the doses of 125 and 250 mg/kg body weight. Maximum stimulating effect was observed during the 2nd (30 min) to 4th (90 min) observation period. The results were also dose dependent and statistically significant ($P < 0.001$) (Fig. 2).

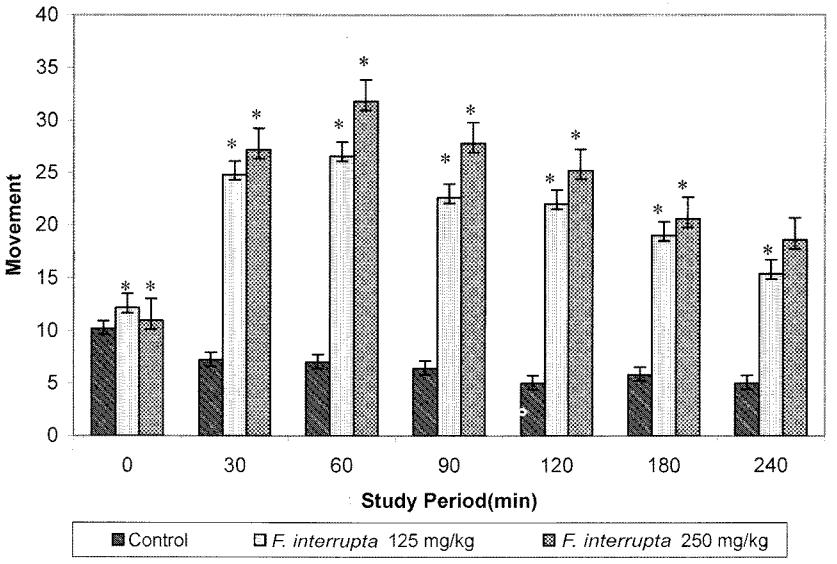

Fig. 2. Effect of the ethanol extract of *F. interrupta* on Hole cross test. $^{*}P < 0.001$ vs control, students *t*-test, values are mean ± SEM. n = 5.

DISCUSSION

The ethanolic extract of *F. interrupta*, at the doses of 125 and 250 mg/kg body weight, showed significant and dose-dependent increase in the acetic acid induced writhing in mice and the results followed a dose dependent response. Intraperitoneal administration of acetic acid causes algesia by liberating noxious endogenous substances including serotonin, histamine, prostaglandin, bradykinin and substance P that sensitize pain nerve endings (Collier *et al.*, 1968; Raj, 1996). Of the prostanoids, mainly prostacycline has been held responsible for the causation of pain following acetic acid administration (Murata *et al.*, 1997). It has been suggested that acetic acid stimulates the vanilloid receptor and bradykinin B_2 receptor in the pathway comprising sensory afferent C-fibers (Ikeda *et al.*, 2001). The reason behind the observed activity of the ethanolic extract of *F. interrupta*, may be due to the effect of the extract in increasing the synthesis and/or release of those endogenous substances or an excitatory effect of the extract on the nerve fibers involved in the pain transmission pathway.

In vivo methods using intact animals are considered to be the best method for investigating the action of drugs on the CNS. The most important step in evaluating drug action on the CNS is to observe the behavior of the test animals. To obtain meaningful results regarding the effect of *F. interrupta* extract on the CNS in mice, a number of methods namely pentobarbitone-induced hypnosis, the open field test and the hole cross test were adopted (Gupta *et al.*, 1971; Takagi *et al.*, 1971). In the pentobarbitone-induced hypnosis test, the extract, at the doses of 125 and 250 mg/kg body weight, delayed the time required for the induction of sleep as compared to the control and also reduced the duration of total sleeping time as compared to the control. The results followed a dose dependant manner and were statistically significant ($P < 0.001$). Pentobarbitone is a barbiturate type of hypnotic agent. When given at appropriate dose, it induces sedation or hypnosis in animals by potentiating the GABA mediated postsynaptic inhibition through an allosteric modification of GABA receptors (Goodman and Gilman, 2001). Substances that have CNS depressant activity either decrease the time of onset of sleep or prolong the duration of sleep or both. Diazepam, used as the positive control in this test, belongs to the benzodiazepine group of anxiolytic and hypnotic agents. In the present study the extract increased the latent period and significantly reduced the total sleeping time as compared to control indicating that the extract of *F. interrupta* might have the inhibitory effect on GABA neurotransmitter or modify the GABA receptor so that reduced the pentobarbitone action on the CNS, which might be attributed to the stimulating action of the cerebral mechanism involved in the regulation of sleep.

Again drugs that act upon the CNS can produce specific physiological and psychological effects. Therefore the extract was also studied using open field and hole cross test in animals and showed an increase in locomotor activity in test animals in dose-dependent manner in both the tests, which suggests an stimulating action on CNS (Guaraldo *et al.*, 2000). Reduce acetic acid induced writhing is an indicator of reduce motor activity (Islam *et al.*, 2003), here the extract also showed a opposite action.

The extract may possess any agent, which might have sympathomimetics action that cause release of the excitatory neurotransmitters from storage vesicles in the CNS and causes the CNS stimulating action, which support our writhing response or reduce the total sleeping time or increased the locomotors activity in the open field and hole cross tests. Indeed other neurotransmitter may be involved in present effects, we did not find any other relations between the chemical components of the plant and neurotransmitters. But all these results supported the finding that *F. interrupta* extract possesses CNS stimulating activity.

Further, pharmacological investigation and bioactivity

guided phytochemical studies are required to find out the active constituent(s) responsible for such activity.

REFERENCES

Collier HO, Dinneen LC, Johnson CA, Schneider C. (1968) The abdominal constriction response and its suppression by analgesic drags in the mouse. *Br. J. Pharmacol.* **32**, 295-310.

Dinda D, Chel G, Das A. (1988) Studied on the relative percentage abundances of the components fatty acids of *Fleurya interrupta. J. Indian Chem. Soci.* **65**, 227-228.

Dinda D, Chel G, Das A, Poria M, Debnath S. (1990) Studied on the relative percentage abundances of the components fatty acids of *Fleurya interrupta. J. Indian Chem. Soci.* **67**, 528.

Goodman, Gilman. (2001) *The Pharmacological Basis of Therapeutics,* edited by Hardman JG, Limbird LE, p. 413, 10th Ed. McGraw-Hill, USA.

Guaraldo L, Chagas DA, Konno AC, Korn GP, Pfiffer T, Nasello AG. (2000) Hydroalcoholic extract and fractions of *Davilla rugosa* Poiret: effects on spontaneous motor activity and elevated plus-maze behavior. *J. Ethnopharmacol.* **72**, 61-67.

Gupta BD, Dandiya PC, Gupta ML. (1971) A psychopharmacological analysis of behavior in rat. *Jpn. J. Pharmacol.* **21**, 293.

Harborne JB. (1984) *Phytochemal methods* (A guide to modern techniques to plant analysis). 3rd ed. Chapman and Hall, London.

Islam MS, Rahman MT, Rouf ASS, Rahman F. (2003) Evaluation of neuropharmacological effects of Rumex maritimus Linn. (Polygonaceae) root extracts. *Pharmazie* **58**, 738-741.

Ikeda Y, Ueno A, Naraba H, Oh-ishi S. (2001) Involvement of vanilloid receptor VRl and prostanoids in the acid-induced writhing responses of mice. *Life Sci.* **69**, 2911-2919.

Koster R, Anderson M, Beer EJ. (1959) Acetic acid for analgesic screening. *Fed. Proc.* **18**, 412-415.

Lorke D. (1983) A new approach to acute toxicity testing. *Arch. Toxicol.* **54**, 275-287.

Murata T, Ushikubi F, Matsuoka T, Hirata M, Yamasaki A, Sugimoto Y, Ichikawa A, ze Y, Tanaka T, Yoshida N, Ueno A, Oh-Ishi S, Narumiya S. (1997) Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. *Nature* **388**, 678-682.

Prain D. (1981) *Bengal plants,* pp. 960-961. Bishen Singh Mahendra Pal Singh Publisher, Dehradun, India.

Raj PP. (1996) Pain mechanisms. In: *Pain Medicine,* pp. 12-24, A Comprehensive Review, first ed. Mosby-Year Book, Inc., St. Louis, USA.

Shilpi JA, Uddin SJ, Rouf R, Billah MM. (2004) Central nervous system depressant activity of *Diospyros peregrina* bark. *Orient. Pharm. Exp. Med.* **4**, 249-252.

Shilpi JA, Ray PK, Uddin SJ. (2005) Analgesic activity of *Amorphophallus campanulatus* tuber. *Fitoterapia* (in press).

Smith AC. (1981) Flora Vitiensis nova: A new flora of Fiji, Lawai, Kauai, Hawaii. *National Topical Botanical Gaeden* **2**, 215-216.

Takagi K, Watanabe M, Saito H. (1971) Studies on the spontaneous movement of animals by the hole cross test: Effect of 2-dimethylaminoethane. Its acylates on the central nervous system. *Jpn. J. Pharmacol.* **21**, 797.

Uddin SJ, Shilpi JA, Alam SMS, Alamgir M, Rahman MT, Sarker SD. (2005) Antidiarrhoeal activity of the methanol extract of the barks of *Xylocarpus molucensis* in castor oil and magnesium sulphate-induced diarrhoea models in mice. *J. Ethnopharmacol.* **101**, 139-143.

Uddin SJ, Shilpi JA, Barua J, Rouf R. (2005) Antinociceptive activity of *Ceriops decandra* leaf and pneumatophore. *Fitoterapia* **76**, 261-263.

Uddin SJ, Shilpi JA, Delazar A, Nahar L, Sarker SD. (2004) Free radical scavengingactivity of some Bangladeshi plant extracts. *Orient. Pharm. Exp. Med.* **4**, 185-193.

Uddin SJ, Shilpi JA, Rahman MT, Ferdous MM, Rouf R, Sarker SD. (2005) Assessment of neuropharmacological activities of *Pandanus foetidus* (Pandanaceae) in mice. *Pharmazie* (In press).

Wagner WL, Herbst DR, and Shomer SH. (1999) Manual of flowering plants of Hawaii, p. 1299, Revised edition, University of Hawaii Press, Honolulu.

Williamson EM, Okpako DT, Evans FJ. (1996) Selection, Preparation and Pharmacological Evaluation of Plant Material. In: *Pharmacological Methods in Phytotherapy Research,* Vol 1, 1st ed, p. 184, John Willey & Sons, England.