Asian Journal of Chemistry Vol. 19, No. 6 (2007), 4452-4458

Evaluation of CNS Activities of Aerial Parts of *Jasminum multiflorum* Andr.

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The ethanol extract of *Jasminum multiflorum* Andr. (EEJM) was tested for possible pharmacological effects on experimental animals. The extract significantly potentiated the sleeping time of mice induced by standard hypnotics *viz.*, pentobarbitone sodium, diazepam and meprobamate in a dose dependent manner. The extract showed significant analgesic properties as evidenced by the significant reduction in the number of writhes and stretches induced in mice by 1.2 % acetic acid solution. Pretreatment with the extract caused significant protection against pentylene tetrazole induced convulsion. The behavioural studies on mice indicate CNS depressant activity of the ethanol extract of *J. multiflorum*.

Key Words: *Jasminum multiflorum*, Sleeping time, General behaviour, Analgesic activity, Anticonvulsant activity.

INTRODUCTION

Jasminum multiflorum Andr. (Sanskrit : Kunda, Bengali : Kundaphul, Hindi: Chemeli) is a large scandent, tomentose shrub with young branches clothed with velvety pubescence, distributed throughout India and is common in the forests of Western Ghats and sub-Himalyan tracts up to 1500 m. Dried leaves of the plant are good for indolent ulcer[1-3]. The flowers are sweet scented, bitter, acrid, refrigerant, laxative cardio tonic, alexipharmic, depurative and digestive. They are useful in vitiated conditious of pitta, inflammation, rheumatism and cephalalgia[1,4,5]. The root of the plant is antidote to cobra venum[2,4,6]. The ethanol extract of *J. multiflorum* (EEJM) showed marked CNS depressant action compared to other extracts of it in preliminary pharmacological screening. However, no work has been reported on the CNS activities of this plant. Keeping this in view, the present study has been undertaken to investigate various CNS activities such as behavioural, sedative-hypnotic, analgesic and anticonvulsant effect of EEJM in mice to substantiate the folklore claim.

EXPERIMENTAL

Fresh aerial parts of *J. multiflorum* were collected from various places of Midnapore district during the month of October-November and were authenticated by Dr. H.J. Chowdhury, Joint Director, Central National Herbarium, Botanical Survey of India, Howrah, West Bengal. A voucher specimen has been preserved in laboratory for future references (DPS 1).

Extraction: Shade-dried powdered and sieved (40 mesh size) plant materials were extracted first with petroleum ether (40-60ºC) and then with ethanol in a soxhlet apparatus. Both the extracts were concentrated to dryness in vacuum. The trace amount of solvent which might be present within the solid mass of respective extracts was removed under vacuum. The yield of petroleum ether and methanol extraction were 2.10 and 7.64 % (w/w), respectively with respect of the dry starting materials. Phytochemical screening of EEJM revealed the presence of cardiac glycoside, steroid, flavonoids and saponin[7-10]. For pharmacological studies, EEJM was dissolved in propylene glycol (PG).

Animals and treatment: Adult albino mice of either sex (20 ± 2 g; Swiss strain) were acclimatized to normal laboratory conditions for 1 week and given pellet diet (Hindustan Lever Ltd., India) and water *ad libitum*. The experiments were performed under the guidance of the Institutional ethical committee.

Safety evaluation: An acute toxicity study relating to the determination of the LD_{50} value was performed with different doses of EEJM in albino mice as per the method described by Litchfield and Wilcoxon[11].

Effect of sleeping time: Mice were divided into 5 groups, each group containing 6 mice. The animals of group I and II received normal saline (5 mL/kg *i.p.*) and vehicle (propylene glycol, 5 mL.kg); groups III, IV and V received EEJM at a low, medium and high dose (35, 50 and 70 mg/kg *i.p.*, respectively). Control and extracts were injected intraperitoneally 0.5 h prior to the administration of pentobarbitone sodium (40 mg/kg, *i.p.*), diazepam (3 mg/kg, *i.p.*) and meprobamate (100 mg/kg, *i.p.*). The sleeping time was noted by recording the interval between the loss and regaining of righting reflex[12].

Analgesic activity: Acetic acid induced writhing (chemical stimulus) method: This method involved *i.p.* injection of freshly prepared 1.2 % acetic acid. The number of abdominal constrictions (writhing) and stretching with a jerk at the hind limbs were counted between 5 and 15 min after administering acetic acid[13-15]. The analgesic effect of EEJM (30, 40 and 50 mg/kg, *i.p.*) was calculated by the percentage inhibition of writhing episode over that of the control group. the results were compared with those of acetyl salicylic acid (68 mg/kg), paracetamol (68 mg/kg) and morphine sulphate (1.15 mg/kg).

Assessment of anticonvulsant activity

Maximum electro shock (MES) induced seizures: EEJM was administered to groups of mice (n = 6) in doses ranging from 75 to 100 mg/kg, *i.p.*, 0.5 h before application of electric shock (42 mA, 0.2 s) using corneal electrodes of an electro convulsiometer (Techno India)[16,17]. The duration of tonic hindleg extension was noted.

Pentylene tetrazole (PTZ) induced seizure: EEJM was administered *i.p.* in varying doses (75-100 mg/kg, *i.p.*) 0.5 h prior to the administration of pentylene tetrazole (PTZ) (80 mg/kg, *i.p.*). The onset of myoclonic spasm, incidence, nature and severity of convulsions and death/recovery were noted. One group received vehicle (propylene glycol, 5 mL/kg, *i.p.*) while other group received diazepam 2.0 mg/kg, *i.p.* as a reference standard. The animals were observed for onset of convulsion up to 0.5 h after PTZ administration. Each group contained 6 animals[18-20].

Behavioural effects: The effects of EEJM (25, 50, 75 mg/kg, *i.p.*) on righting reflex, pinna reflex, corneal reflex, awareness, grip strength, touch and pain responses on mice were observed by conventional methods. Chlorpromazine (5 mg/kg, *i.p.*) was used as a reference drug[21,22].

Statistical analysis: Results are expressed as mean ± SEM. Anova followed by Dunnett's 't' test[23] was performed as a post hoc test of significance taking vehicle treated animals as control. $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Safety evaluation: Acute toxicity test in mice established the LD_{50} of EEJM to be 440 mg/kg, *i.p*.

Effect on sleeping time: 3 Dose of EEJM (30, 50 70 mg/kg, *i.p.*) lengthened. Signifcantly the sleeping time induced by standard hypnotics *viz.*, petobarbitone (58, 97.4 and 138.2 %, respectively), meprobamate (29.6, 52.1 and 84.4 %, respectively) and diazepam (54.3, 95.4 and 175.5 %, respectively) (Table-1).

Analgesic activity: As can be seen in Fig. 1, EEJM significantly reduced the number of writhes and stretches induced in mice by 1.2 % solution of acetic acid, with a dose of 30 mg/kg, *i.p.* the percentage of protection 70.5 %. This dose dependant effect reached 88.2 % with a dose of 40 mg/kg, *i.p.* and protection was complete with a dose of 50 mg/kg, *i.p.* Analgesic compounds acetyl salicylic acid (68 mg/kg), morphine sulphate (1.15 mg/kg) and paracetamol (68 mg/kg) gave 60.15, 70.12 and 61.43 %, protection, respectively.

Anticonvulsant activity: The duration of tonic hind leg extention in mice treated with vehicle was 15.69 ± 1.35 s whereas mice treated with EEJM (50, 70 and 100 mg/kg, *i.p.*) exhibited hindleg extension for 14.65 ±

TABLE-1
EFFECT OF EEJM ON SLEEPING TIME (min) INDUCED BY PENTOBARBITONE, DIAZEPAM AND MEPROBAMATE IN MICE

Treatments	Pentobarbitone (40 mg/kg, *i.p.*)	Sleeping time (min) induced by meprobamate (100 mg/kg, *i.p.*)	Diazepam (3 mg/kg, *i.p.*)
Control (PG, 5 mL/kg, *i.p.*)	419. ± 0.47	62.9 ± 0.61	71.4 ± 0.90
EEJM (30 mg/kg, *i.p.*)	66.4 ± 1.09*	81.5 ± 1.22*	110.2 ± 1.40*
EEJM (50 mg/kg, *i.p.*)	82.7 ± 2.10*	95.7 ± 1.95*	139.5 ± 3.05*
EEJM (70 mg/kg, *i.p.*)	99.8 ± 2.17*	116.0 ± 2.07*	196.7 ± 3.75*

Value are mean ± SEM from 6 animals in each group; statistical analysis done by Anova followed by post hoc test of significance, Dunnett's 't' test. *p < 0.05 *vs.* vehicle control. PG: propylene glycol; i.p.: intraperitoneal.

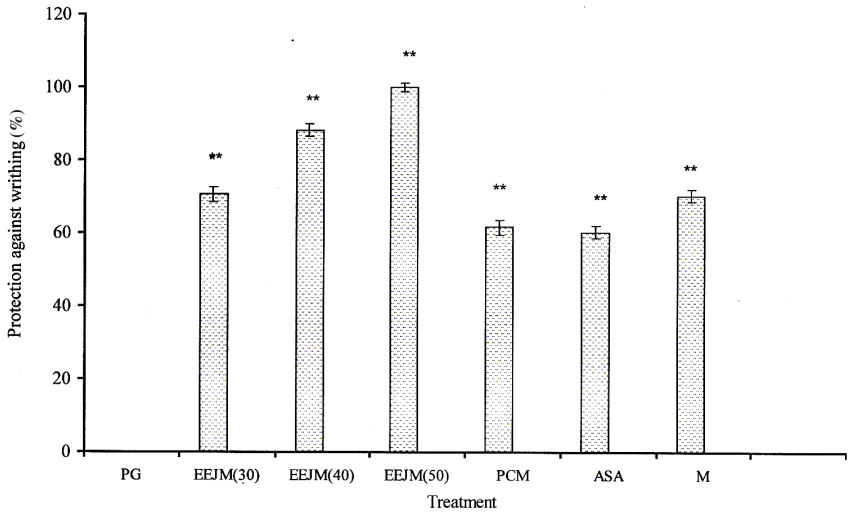

Fig. 1. Influence of EEJM (30, 40 and 50 mg/kg *i.p.*) on the writhing and stretching induced in mice by 1.2 % v/v acetic acid (writhing test). The activity was compared with paracetamol (PCM) (68 mg/kg *i.p.*) acetyl salicylic acid (ASA) (68 mg/kg *i.p.*) and morphine sulphate (M) (1.15 mg/kg *i.p.*). Values are mean ± SEM from 6 animals in each group. *p < 0.01, ** p < 0.001 *vs.* control

1.17, 18.35 ± 1.82 and 16.41 ± 1.41, respectively. In pentylene tetrazole-induced seizure, the animals treated with vehicle, clonic convulsion appeared 100.4 ± 10.6 s after PTZ and all animals died after seizures. The EEJM significantly dose dependently inhibited the onset and incidence of convulsion. The convulsions were completely abolished by the dose of 100 mg/kg, *i.p.* EEJM in a dose of 50 mg/kg, *i.p.* exhibited seizures in 68.5 % in mice and all animals exhibiting seizures died within 0.5 h. No mortality was observed in the groups treated with EEJM 75 and 100 mg/kg even after 24h. Diazepam (2 mg/kg) inhibited seizures completely (Table-2).

TABLE-2
ANTICONVULSANT EFFECT OF EEJM (50, 75, 100 mg/kg, *i.p.*) ON PENTYLENE TETRAZOLE INDUCED SEIZURES IN MICE

Treatment	Onset of clonic convulsion (s) (Mean ± SEM)	Incidence of convulsion (%)	Nature and severity	Death/ recovery (%)
Control (PG, 5 mL/kg, *i.p.*)	70.2 ± 4.3	100.0	Major jerking	All died
EEJM (50 mg/kg, *i.p.*)	230.5 ± 13.8*	68.5	Minor jerking	68.5 died
EEJM (75 mg/kg, *i.p.*)	375.4 ± 31.2*	28.7	Minor jerking	No death
EEJM (100 mg/kg, *i.p.*)	A	0	Nil	No death
Diazepam (2mg/kg, *i.p.*)	A	A	Nil	No death

*$p < 0.001$ compared to vehicle treated group (Anova followed by Dunnett's 't' test); n = 6, A = absence of convulsion; PG: propylene glycol; i.p.: intraperitoneal.

Behavioural effects: The results obtained from general behavioural profiles are shown in Table-3. It was noted that EEJM depressed awareness and alertness, touch and pain responses, grip strength, altered righting, pinna and corneal reflexes when compared to the control (propylene glycol, 5 mL/kg). However, chlorpromazine hydrochloride (standard) product a significant depression of these responses in comparison with EEJM.

Conclusion

Pentobarbitone, diazepam and meprobamate were used to induce sleep in this study. Benzodiazepines are believed to act at specific binding sites which are closely linked to γ-aminobutyric acid (GABA) receptors, the binding of benzodiazepines enhancing GABA-ergic transmission. Although the cause of prolongation of diazepam-induced sleeping time is not known, the enhancement of GABA-ergic transmission might be related to its

sedative activity. Prolongation of pentobarbitone induced sleeping time might be due to tranquilizing action as well as CNS depressant action. Although the exact mechanism responsible for the sedation action of meprobamate is not clear, it may be due to CNS depressant action or due to enhancement of GABA-ergic transmission[24-26]. EEJM potentiated significantly the duration of pentobarbitone, diazepam and meprobamate-induced sleep in mice, suggesting probable tranquilizing action as well as CNS depressant action[27].

TABLE-3
EFFECT OF EEJM ON BEHAVIOURAL PROFILES IN MICE

Treatments	AR	TR	PR	RR	Pr	CR	GS
Control (PG, 5 mL/kg, *i.p.*)	0	0	0	0	0	0	+
Chlorpromazine (5 mg/kg, *i.p.*)	4+	4+	4+	4+	4+	4+	4+
EEJM (30 mg/kg, *i.p.*)	2+	2+	2+	2+	+	+	+
EEJM (50 mg/kg, *i.p.*)	3+	3+	3+	3+	2+	3+	3+
EEJM (70 mg/kg, *i.p.*)	4+	4+	4+	4+	3+	4+	4+

AR = Awareness response; TR = Touch response; PR = Pain reflex; RR = Righting reflex; Pr = Pinna reflex; CR = Corneal reflex; GS = Grip strength. Key for scoring: 0, no effect (normal); +, slight depression; 2+ moderate depression; 3+ strong depression; 4+ very strong depression, *i.p.*: intraperitoneal. PG: propylene glycol. Number of animals used for each group (n = 6). EEJM values are significant ($p < 0.05$) *vs.* control.

Mazumder *et al.*[26] found that analgesic activity of *Cassia fistula* is probably mediated by inhibition of a post synaptic specific sensitive mechanism either by depleting endogenous levels of nor epinephrine *via* dopamine-β-hydroxylase inhibition or by blocking nor epinephrine effects at the receptor level. Analgesic and anticonvulsant activities can also be mediated by other mechanisms. The increase of brain serotonin and GABA level is responsible for analgesic and anticonvulsant activities[18,26,27].

Gupta *et al.*[18] estbalished that inhibition of the touch response, righting reflex and grip strength is probably produced due to a pronounced CNS depressant action. Reduction of pinna reflex and awareness may be due to synapses block of the afferent pathway or due to overall CNS depressant action[29]. In this study, the mechanism whereby EEJM depressed awareness, touch and pain responses, righting reflex, pinna reflex, corneal reflex and grip strength may also be due to synapses block of the different path way or by overall CNS depressant action. Since various steroids have been reported to possess analgesic[29,30] and anxiolytic[31] activities, the analgesic effect of EEJM in mice might be due to the presence of such compounds. Similarly, the anticonvulsant effect of EEJM might be due to the presence of saponin[32,33].

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(*Received*: 29 May 2006; *Accepted*: 21 April 2007) AJC-5578