RESEARCH PAPER

Received 31st March, 1997
Accepted 15th July, 1997
Indian J. Pharm. Sci., 1997, 59(5) pp. 240-245

CNS activity of Petroleum Ether extract of *Vitex Negundo* Linn in mice

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The dried leaves of *Vitex negundo* was extracted with petroleum ether. The active fraction of the extract was separated by column chromatographic technique, and the Acute toxicity studies were done using Swiss albino mice of either sex. Three dose levels (125, 167, 250 mg/kg b.w.) were chosen to study analgesic, anti-convulsant and sedative-hypnotic activities using Swiss albino mice of either sex. The leaf extract potentiated the sedative-hypnotic effect of pentobarbitone and diazepam significantly ($P \leq .001$) in a dose dependent manner, while it did not produce sleep in mice itself. The extract showed significant analgesic activity and prolonged the analgesia produced by morphine and pethidine, whereas, the anti-convulsant activity against strychnine and leptazole was found only at high dose levels (250 mg/kg b.w.).

VITEX negundo Linn. (Fam. Verbenaceae) is a widely grown shrub in India. In the traditional system of medicine, different parts of the shrub were used to treat a variety of disease conditions[1]. The plant is well known as Nishinda in the northern states while Vellai Nochi in the southern states. The decoction of the leaf was considered tonic and vermifuge, and given with long popper in catarrhal fever with heaviness of head and dullness of hearing[2,3]. The leaf extract has been reported to be used in Ayurvedic[4] and Unani[5] system of medicine and in Herbal Eye Drop preparations[6]. It also has insect repellent activity against a wide range of organisms[7] and produces antibacterial and antifungal activities[8]. It is used in Rheumatoid arthritis, and was shown to posses anti inflammatory activity[9] and antihistaminic activity in rats, bronchial relaxant effect and mast cell stabilizing effect[10]. The leaves contain essential oils and two new iridoid glucosides Negundoside and Nishindaside were isolated and structures determined[11]. The present study is focussed on the evaluation of CNS activity of *V. negundo* in mice.

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MATERIALS AND METHODS

Preparation of extract

The cut, dried leaves were obtained from the local supplier (United Chemicals and Allied Products, Calcutta, India) and was identified in the Pharmacognosy laboratory of our department. Petroleum ether (40-60°), n-hexane, chloroform, methanol and silica gel for column chromatography (60-120 mesh size) (s.d. fine chem.), silica gel for thin layer chromatography (Sisco lab. Bombay).

The cut, dried leaves were packed in high quality filter paper, cotton plugged and extracted with petroleum ether. After sufficient amount of the extract was collected, dried under vacuum. The crude extract was then subjected to thin layer chromatography using hexane : chloroform (3 : 2) as the mobile phase. Three components were identified, having the Rf value 0.16, 0.29, 0.90 respectively. Sufficient amount of the separate fraction were collected by column chromatographic technique. This was then taken for further study.

Acute toxicity studies

The LD_{50} value of petroleum ether extract of *Vitex negundo* was estimated by the 'Acute Toxicity Test'. The purpose of an acute toxicity test is to determine the nature and extent of untoward reactions which might follow the administration of a single dose (or an overdose) of the drug. Petroleum ether extract of *v. negundo* was dissolved in groundnut oil. Albino mice of either sex weighing between 20 to 25 g were used. Animals were allowed free access to standard pellet food (Hindustan Lever) and drinking water *ad libitum*. Animals were not provided food for last 18 hours before the commencement of experiments. The experiment was performed with eight groups containing 10 animals each. The doses of the extract dissolved in groundnut oil were administered intraperitoneally to different groups in increasing dose levels of 250, 500, 750 1000, 1250, 1500, 1750 mg/kg b.w. The animals were observed once in half an hour for next 4 h and the once in 25 h to find out the percent mortality.

The LD_{50} value was derived using the Litchfield and Wilcoxon method[12]. In the interpretation of the toxicity (LD_{50}) the observed percentage morality was converted to probit by referring to the appropriate table[13]. The values thus obtained were plotted against the corresponding log dose. Before plotting the percentage for the zero and hundred were corrected. Results were fitted with straight line after regression analysis of the probits. The log dose corresponding to probit 5 was found to be the 2.93 and its antilog is 851.13. Therefore, the LD_{50} value of the petroleum extract of *V. negundo* in mice is 851.13 mg/kg body weight. (Fig.1)

Effect of the Extract of *Vitex negundo* Linn on the Sleeping time induced by Sedatives in Mice.

Diazepam (calmpose, Ranbaxy lab., India), Pentobarbitone (May and Baker, India), groundnut oil, and petroleum ether extract of *Vitex negundo* were used in these experiments.

Albino mice of either sex weighing 20-25 g were used. The animals were fed standard pellet diet and given tap water *ad libitum*. The experiments were performed in a quiet room with an ambient temperature 22 ± 2°. The mice were placed in specially designed cages so as to acclimatize them to the environment, and to avoid behavioural changes resulting from circadian rhythm. The animals were divided into 13 groups, each group containing ten mice only. The first seven groups were used as saline control, vehicle control, standard drug control and three different doses of the extract dissolved in groundnut oil were administered intraperitoneally. In the remaining six groups three doses of the extract were administered intraperitoneally 15 min prior to the administration of either pentobarbitone or diazepam.

The criterion for sleep in mice was loss of righting reflex and the moment the mice were able when placed on their backs, to right themselves three times consecutively. The unpaired student's 't' test was applied to evaluate the statistical significance of the data obtained.

Anticonvulsant Activity[15]

The mice were divided into 20 groups with 10 mice in each group, weighing 20-25 g. All the injections were given intraperitoneally. The groups were divided into set A and B, each set containing 10 groups, set A was used for strychinine and set B for leptazole. The extract was administered at 3 dose levels (125, 167, 250 mg/kg b.w.) prior to the administration of strychnine (2 mg/kg b.w.) and leptazole (80 mg/kg b.w.) respectively. The percentages mortality were noted for 15 min and 30 min pretreatment of the extract.

Analgesic Activity[16,17]

All the mice were divided into 13 groups, each group containing ten mice, weighing 20-25 g. The analgesic action was studied by hot plate method. The effects wee observed at 15 and 30 min after

Table 1 : Effect of petroleum ether extract of *V. Negundo* on sleeping time induced by pentobarbiton and diazepam

Reference drugs(s)	Dose mg/kg b.w.	Ref. Drug Saline	Ref. Drug + Vehicle	Reference drug(s) and extract at different doses (mg/kg b.w.)		
				125	167	250
Pentobarbitone	4	27.8±0.45	29±2.67	32±2.90	43±2.48*	63.3±5.1*
Diazepam	6	35±0.6[3]	38.3±0.85	42±1.08*	63±1.29*	73±0.645*

*Highly significant

Table 2 : Effect of petroleum ether extract of *V. Negundo* on strychnine induced convulsion in mice

	Drugs Dose (mg/kg b.w.)	Percentage mortality	
		15 min Pretreatment	30 min Pretreatment
1.	Saline (5 ml/kg b.w) + Strychnine (2)	100	100
2.	Vehicle (5ml/kg b.w.) + Strychnine (2)	100	100
3.	Extract (125) + Strychnine (2)	100	100
4.	Extract (167 + Strychnine (2)	100	80
5.	Extract (250) + Strychnine (2)	80	60

intraperitoneal administration of the drug(s). The reaction time was taken as the interval extending from dropping the animal on the hot surface until the instant the animal licked the hind paw. All other signs of discomfort were disregarded. The temperature of the hot plate was maintained at 55 ± 0.5°. A cut-off reaction time of 30 sec (when the mouse made no response) was used in order to avoid tissue injury. If the animal could not respond by that time it was considered to be completely analgesia. The extract was administered at 3 dose levels (125, 167, 250 mg/kg b.w.) 15 min prior to the administration of morphine hydrochloride (5 mg/kg b.w.) or pethidine (10 mg/kg b.w.) respectively. The remaining groups were used as control for saline, vehicle, vehicle + standard drug, extract only. The unpaired students "t" test was applied to evaluate the statistical significance of the data obtained.

RESULTS AND DISCUSSION

Results are summarised in tables 1-5. The table indicates that petroleum ether extract of *Vitex negundo* itself dose not have any sedative-hypnotic action but it can potentiate the sedative - hypnotic action of drugs such as diazepam and pentobarbitone. The extract at doses varying from 125, 167, 250 mg/kg b.w. increased sleeping time induced by

Table 3 : Effect of petroleum ether extract of *V. negundo* on leptazole induced convulsions in mice

	Drugs dose (mg/kg b.w.)	Percentage mortality	
		15 min Pretreatment	30 min Pretreatment
1.	Saline (5 ml/kg b.w.) + Leptazole (80)	80	60
2.	Vehicle (5 ml/kg b.w.) + Lotazole (80)	80	60
3.	Extract (125) + Laptazole (80)	60	60
4.	Extract (167) + Leptazole (80)	60	40
5.	Extract (250) + Leptazole (80)	40	40

Table 4 : Analgesic activity of petroleum ether extract of *vitex negundo* in mice by hot plate method

	Drugs	onset of reaction (after 15 min.)
1.	N. Saline	4.5 Sec ± 0.65
2.	Vehicle control (5 ml/kg b.w.)	5 Sec ± 0.2
3.	Extract (125 mg/kg b.w.)	7.5 Sec ± 20.52*
4.	Extract (167 mg/kg b.w.)	11.5 Sec ± 0.29*
5.	Extract (250 mg/kg b.w.)	13.5 Sec ± 0.07*

* - $P \leq 0.01$

diazepam and pentobarbitone significantly (P<.001) in a dose dependent manner. Although, actual cause of prolongation of diazepam-induced sleeping time is not known but enhancement of GABAergic transmission[18] might be related for its prolongation of sleeping time. The petroleum ether extract prolonged the pentobarbitone-induced sleeping time in mice probably by its CNS depressant action.

Table 2 and 3, indicate that leptazole-or strychnine-induced convulsions in mice were not prevented by the 15 min pretreatment of the extract, however, 30 min pretreatment of extract at a dose of 250 mg/kg body weight caused protection against tonic extensor reflexes produced by leptazole or strychnine. The anticonvulsant effect of the extract at high dose levels may be due to the blocking of the multineuronal pathway in the spinal cord with the minimal effect of monosynaptic pathways[19] and also may be due to alteration of GABAergic transmission.

Table 4 and 5 exhibits that the petroleum ether extract produced significant effect on pain sensation. From the narcotic analgesic study it is evident that the extract of *Vitex negundo* produced analgesia alone and potentiated the analgesic effect of morphine or pethidine significantly (P.001) in mice. As it was observed that the extract was found to

Table 5 : Effect of the extract on analgesia induced by morphine and pethidine by hot plate method

	Drugs	on set of reaction (in sec)
1.	Vehicle + Morphine (5 ml/kg b.w.) (5 mg/kg b.w.)	19.3 ± 4.9
2.	Extract (125 mg/kg b.w.) + Morphine (5 mg/kg b.w.)	23.5 ± 1.94*
3.	Extract (167 mg/kg b.w.) + Morphine (5 mg/kg b.w.)	26.8 ± 1.32*
4.	Extract (250 mg/kg b.w.) + Morphine (5 mg/kg b.w.)	28.8 ± 0.25*
5.	Vehicle + Pethidine (5 ml/kg b.w.) (10 mg/kg b.w.)	20.3 ± 1.7
6.	Extract (125 mg/kg b.w.) (+ Pethidine (10 mg/kg b.w.)	21.8 ± 2.06
7.	Extract (167 mg/kg b.w.) + Pethidine (10mg/kg b.w.)	24.3 ± 1.25*
8.	Extract (250 mg/kg b.w.) + Pethidine (10mg/kg b.w.)	26.8 ± 0.8*

*p ≤ 0.01

have central effects, the petroleum ether extract significantly altered the threshold to pain sensation, thereby producing a prominent analgesic effect.

It is evident from the observations that the petroleum ether extract of *Vitex negundo* exhibited sedative - hypnotic and analgesic activity in a dose dependent manner, whereas, the anticonvulsant activity was observed only at a high dose. The active component of the petroleum ether extract may be considered as a potent CNS depresent agent. Further work will enlighten the exact mechanism of action of the extract.

ACKNOWLEDGEMENTS

The authors thank the Registrar, Jadavpur University for the research facilities provided and to UGC, New Delhi, for providing financial assistance.

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