

Research Article

Some Central Nervous System Activities of *Nerium Oleander* Linn (Kaner) Flower Extract

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Abstract

Purpose: The purpose of the study was to evaluate the activity of 50 % hydroalcohol flower extract of *Nerium oleander* Linn. on the central nervous system (CNS) of mice.

Methods: The effect of the 50 % hydroalcohol extract of *N. oleander* flowers at dosage levels of 100 and 200 mg/kg p.o. on the locomotor activity of mice was measured with an actophotometer while muscle relaxant activity was evaluated on rotarod apparatus. Also, the anticonvulsant activity of the extract in mice (following maximal electroshock and pentylenetetrazol-induced convulsion) as well as the potentiation of pentobarbital-induced sleep were determined using standard procedures. Diazepam was used as the reference standard.

Result: The extract (at doses of 100 and 200 mg/kg) significantly reduced ($p < 0.01$) spontaneous locomotor activity and also potentiated pentobarbital-induced sleep. At the higher dose (200 mg/kg) the extract showed 66 % protection against electroshock-induced convulsions while the lower dose (100 mg/kg) produced a significant reduction ($p < 0.01$) in pentylenetetrazol (PTZ)-induced convulsions.

Conclusion: The ethanol extract of *Nerium oleander* flowers has anticonvulsant activity in an experimental animal model.

Keywords: *Nerium oleander* extract, CNS depression, Locomotor activity, Anticonvulsant activity.

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INTRODUCTION

Nerium oleander Linn. (Kaner) belongs to the family Apocynaceae. It is a large glabrous evergreen shrub that produces milky juice. It is native to Iran, the Mediterranean region, as well as India. The leaves are in pairs of three, shortly stalked, coriaceous, 10 - 15 cm long, linear lanceolate with dark green colour. The flowers are salver-shaped pink or white without any fragrance [1].

In the traditional medicine system, parts of this plant are used for the treatment of various human ailments. The leaf is used as a cardiogenic, diuretic, anti-bacterial in cutaneous eruptions, and is also effective against snake-bites; the root is used for curing different types of cancers, ulcers and leprosy. The root-bark is used specifically against ring worm and the aqueous extracts of the leaves, branches, roots and flowers are toxic to certain insects [2]. Several phytochemicals have been identified in various parts of the plant and they include mainly cardiogenic glycosides, terpenoids and steroids [1].

A recent study has established that the hot aqueous extract of *N. oleander* leaves (Anvirzel) inhibits fibroblast growth factor-2 and also has anti-tumor activity [3]. Another study demonstrated that the leaf extract has antibacterial and sedative-hypnotic activities and also affects behavioral pattern in mice [4]. The dried and fresh flowers of the plant have been reported to exhibit potent antiinflammatory activity against carrageenan hind paw edema model in mice without inducing any gastric damage [5].

Although the leaves and the root have been the subject of several studies, there has been little investigation of the flowers. The objective of this study, therefore, was to investigate the anticonvulsant activities of the 50 % hydroalcohol flower extract.

EXPERIMENTAL

Drugs, chemicals and equipment

Diazepam (Sigma Aldrich) was used as a standard CNS depressant and anticonvulsant. Pentylene-tetrazol and pentobarbital (both from Sigma Aldrich), ethanol (Changshu Yangyuan Chemicals) China, petroleum ether (40 – 60 °C, Merck), rotary evaporator (Heidolph Schwabach, Germany), freeze dryer (Vis Tis Advantage, 2.0 EL-85), actophotometer (Imcorp, Ambala, India), rotarod (model KI 9616, Ambala, India), electroconvulsimeter (Inco, Ambala, India), and grinding mill (Philips- HL1606 Gurgaon, India) were also used in the study.

Plant material

N. oleander flowers were collected from Kota district of Rajasthan in India. The flowers were authenticated by Dr SN Sharma, taxonomist, Botany Division, Indian Institute of Integrative Medicine (IIIM), Jammu, India. A voucher specimen (CDR accession no. 21869) was deposited at the crude drug repository of the herbarium of IIIM.

Preparation of extract

The flowers (2 kg) were shade-dried and coarsely powdered with a grinding mill. The powder was extracted with petroleum ether (40 – 60 °C) to defat it and then macerated with ethanol:water (1:1) with constant stirring (1200 rpm, 3 h). The solvent incorporating the extractives was filtered and the marc pressed to squeeze out residual extractives. This process was repeated thrice to achieve complete extraction. The extracts obtained during the three cycles were combined and reduced to 1/8th of its original volume in a rotary evaporator at 45 °C and then lyophilized in a freeze dryer to obtain a yield of 11 %w/w.

Animals

Male Swiss albino mice (8 - 12 weeks old) weighing 18 - 20 g were purchased from the Central Animal House, IMTECH, Chandigarh, 55/1999/ CPCSEA. The animal study protocol was approved by the Institution Animals Ethics Committee (IAEC) of ASBASJSM College of Pharmacy, Bela (Ropar) Punjab (approval no. ASCB/IAEC/02/10/005). The animals were housed under standard laboratory conditions (23 ± 1 °C, and 55 ± 10 %RH, 12/12 h light/dark cycle) and fed with standard pellet diet (M/s Ashirwad Industries, Mohali) and purified water *ad libitum*.

Acute toxicity study

Determination of maximum tolerable dose was performed according to OECD (Organization for Economic Corporation and Development) guideline 423 (11) [6]. The study was performed at graded doses of 5, 50, 300 and 2000 mg/kg p.o. using 3 female mice (18 – 22 g) at each dose level. The mice were deprived of food 3 - 4 h prior to the experiment and thereafter individually administered the extract. Each animal was continuously monitored during the first 30 min, then on hourly basis for the next 4 h, and subsequently, at four hourly interval. Finally, they were placed under observation for 14 days to monitor any abnormal signs and symptoms depicting toxicity. The animals were then humanely killed by a high inhalation dose of diethyl ether and observed for any changes in skin and fur, eyes, mucous membrane (ear), respiratory, circulatory, autonomic and central nervous systems, as well as somatosensory activity and behavioral pattern. Attention was given to phenomena such as tremors, convulsions, salivation, diarrhoea, lethargy, sedation, hypnosis and coma [6].

Assessment of spontaneous motor activity

The animals were divided into four groups of 6 mice each. Group I served as control and

received normal saline (1 ml/100 g body weight); Group II received the reference standard (diazepam, 2 mg/kg i.p.) while Groups III and IV received 100 and 200 mg/kg p.o. of the extract, respectively. To evaluate spontaneous motor activity, each mouse was introduced into an actophotometer and its score of locomotor was measured after a period of 10 min [7].

Potentiation of pentobarbital-induced hypnosis

The animals (excluding those used in the preceding experiment) were divided into three groups (n = 6). Group I was administered pentobarbital (50 mg/kg, i.p.); Groups II and III received 100 and 200 mg/kg of the extract orally, respectively, and then were injected with pentobarbital (50 mg/kg, i.p.) 2 h later. The time elapsed between loss and recovery of the righting reflex was noted and taken as sleeping time [8].

Evaluation of muscle relaxant activity

Before performing this experiment, the fresh mice were trained to remain on rotarod apparatus (with the rod rotating at a speed of 25 rpm) for 3 min. After training, the mice were divided into three groups (n = 6). Group I served as control, Group II as reference standard (diazepam, 4 mg/kg i.p.) while Groups III and IV were administered the extract (100 and 200 mg/kg p.o., respectively). The effect on motor coordination was assessed with the rotarod apparatus as the fall-off time from the rotarod [7].

Determination of anticonvulsant activity Pentylentetrazol (PTZ)-induced convulsion test

Four groups of mice (n = 10) were used. Group I was administered the vehicle, i.e., normal saline (1 ml/100g body weight) and served as control, Group II received reference standard (diazepam, 2 mg/kg, i.p.) while Groups III and IV were administered 100 and 200 mg/kg, p.o., respectively, of the

extract. Two hours later, PTZ was administered (60 mg/kg, i.p.) to all four groups. The animals were observed for 30 min and the onset and duration of convulsion noted [8].

Maximum electroshock convulsion test

Four groups of mice were used in this test. Group I ingested normal saline (1 ml/100 g) and served as control; Group II received the reference drug (diazepam, 2mg/kg, i.p.) while Groups III and IV were administered the extract (100 and 200 mg/kg, p.o., respectively). Two hours later, they were given a current of 12 mA 50 Hz for 0.2 sec in an electroconvulsimeter via an ear electrode. The onset of convulsion and duration of tonus were noted. Complete protection (100 %) against convulsion is achieved only when hind limb tonic extension is completely abolished [8].

Statistical analysis

All the data obtained were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's test or Student 't' test (Graph-pad Prism statistical software). $P < 0.05$ was considered statistically significant.

RESULTS

Acute toxicity of extract

The maximum tolerable dose of the extract via oral administration was 2000 mg/kg body weight.

Table 1: Effect of *N. oleander* (NO) flower extract on spontaneous motor activity in mice (n = 6)

Treatment (mg/kg, p.o.)	0 h	1h	2 h	3 h
Vehicle	198.7 ± 3.3	209.3 ± 4.0	205.3 ± 4.8	208.5 ± 4.2
Diazepam	195.5 ± 3.0	10.2 ± 2.7 [†]	24.2 ± 2.2 [†]	26.12 ± 2.8 [†]
NO (100 mg/kg)	202.3 ± 3.6	194.0 ± 4.3 [#]	28.3 ± 4.4 [†]	22.7 ± 4.0 [†]
NO (200 mg/kg)	203.0 ± 3.9	193.0 ± 10.1 [#]	33.5 ± 2.6 [†]	29.3 ± 4.4 [†]

Statistically significant at [#] $p < 0.05$ or, [†] $p < .001$

Extract effect on spontaneous motor activity

Table 1 shows the effect of the extract on spontaneous locomotor activity. Compared with the control group, a small but significant reduction ($p < 0.05$) in locomotor activity was observed after 1 h. However, much higher reduction in locomotor activity by the reference standard, diazepam, was found. Both the extract and standard effected very drastic reduction ($p < 0.001$) in locomotor activity after 2 and 3 h, respectively.

Potentiation of pentobarbital-induced hypnosis

Pentobarbital did not modify the latency to induce sleep after extract administration. However, as Fig 1 shows, the former increased the duration of extract-induced hypnosis significantly ($p < 0.05$ and $p < 0.001$) at doses of 100 and 200 mg/kg, respectively.

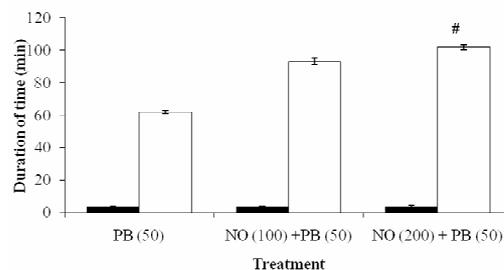


Fig 1: Potentiating of *N. oleander* flower extract-induced hypnosis by pentobarbital in mice. **Key:** PB (50) = pentobarbital 50 mg/kg, i.p.; NO (100) = extract 100 mg/kg; NO (200) = extract 100 mg/kg induced hypnosis; ■ = latency and □ hypnosis; [#] $p < 0.001$; [†] $p < 0.01$

Muscle relaxant activity of extract

The animals did not show any motor uncoordination after administration of the extract.

Effect of extract on pentylenetetrazol-induced convulsions

Table 2 shows that the extract increased the onset of pentylenetetrazol-induced seizures but reduced the duration of the convulsions. The increase in the onset of seizure was statistically significant, $p < 0.01$ and $p < 0.05$ for extract doses of 100 and 200 mg/kg, respectively, and highly significant ($p < 0.001$) for the standard drug, diazepam; with regard to their duration reduction effect, the difference was statistically significant at $p < 0.01$ for both doses. However, the clonus phase was completely abolished only at the lower dose of the extract and also with diazepam.

Table 2: Effect of *N. oleander* (NO) flower extract on pentylenetetrazol (PTZ)-induced seizures in mice (n = 10)

Treatment	Onset of convulsion (min)	Duration of convulsion (min)
Vehicle (control)	1.02 ± 0.11	28.9 ± 0.2
Diazepam	0 ± 0 [†]	0 ± 0 [†]
NO (100mg/kg)	4.99 ± 0.17 [#]	7.0 ± 0.1 [#]
NO (200mg/kg)	1.60 ± 0.13 [*]	16.2 ± 0.1 [#]

[†] $P < 0.001$; [#] $p < 0.01$; ^{*} $p < 0.05$

Effect of extract on maximal electroshock convulsions (MEC)

As Table 3 indicates, the higher dose of the extract (200 mg/kg) was more effective than the lower dose in lowering MEC. However, while the lower dose failed to provide protection against convulsion, the higher dose provided 60 % protection, compared with 100 % for the reference standard, diazepam.

Table 3: Effect of *Nerium oleander* (NO) flower extract on maximal electroshock convulsions (MEC, n = 10)

Treatment	Duration of tonic hind limb extensor (sec)	Incidence of convulsions	Protection (%)
Vehicle (control)	14.8±1.0	10/10	0
Diazepam (2)	0±0 [†]	0/10	100
NO (100mg/kg)	11.3±0.7 [*]	10/10	0
NO (200mg/kg)	2.3±0.6 [#]	04/10	60

[†] $P < 0.001$; [#] $p < 0.01$; ^{*} $p < 0.05$

DISCUSSION

Acute toxicity data indicate that the maximum tolerable dose of *N. oleander* flower extract in mice is 2000 mg/kg body weight, suggesting that the extract is not highly toxic.

The reduction in locomotor activity following the administration of the extract implies that it exerted a depressive effect on the CNS [9]. It has been established that increase in the concentration of gamma-amino butyric acid (GABA) may lead to CNS depressant effect [10]. This led to further exploration of the effect of the extract on activities responsible for increase in GABA concentration, such as potentiation of hexobarbital-induced hypnosis, motor coordination, anticonvulsant activity. The fact that extract activity was potentiated by pentobarbitone in induced hypnosis suggest a GABA-mediated effect on the CNS since CNS depressants extend barbiturate sleeping time [11].

It is known that sedative-hypnotic drugs induce their effect on the GABAergic system in the brain and inhibition of neuronal output could be facilitated by GABA (an inhibitory neurotransmitter) release [12]. Loss of righting reflex induced by phenobarbital is

potentiated by GABA agonist (muscimol and 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) and inhibited by GABA antagonist (bicucullin); the activation of GABA receptor partially mediates the sleep response [13]. It is thus plausible to assert that the sedative effect of the extract is due to the facilitation of GABAergic transmission. The findings in respect of the extract of *N. oleander* flower are in agreement with those reported for some other plants (*Rauwolfia canescens* [14] and *Carissa edulis* [15]). The fact that the extract had no effect on motor coordination also implies that it exerts significant CNS depressant effect through GABA receptor stimulation.

The test on the anticonvulsant activity of the extract is in effect a measure of its stimulatory effect on GABA receptor. Two different anticonvulsant animal models were used: MES- and PTZ-induced convulsion models. The extract showed anticonvulsant activity in PTZ-induced convulsion model and the higher dose of the extract (200 mg/kg) was more effective than the lower dose in lowering MEC. PTZ-induced activation of T-type Ca^{2+} channels of the thalamic region produces *petit mal* seizures [16]. Compounds that are effective in PTZ-induced seizure models are effective against *petit mal* type of epilepsy [17]. *Petit mal* seizures can also be prevented by drugs that enhance GABA_A-BZD receptor mediated neurotransmission such as benzodiazepines and phenobarbitone [18]. Protection against hind limb tonic extension also shows the capability of the extract to prevent seizure discharge within the brain stem seizure substrate [19], suggesting that the extract may be useful in the treatment of generalized tonic-clonic seizures.

CONCLUSION

On the basis of results obtained, the hydroalcohol extract of *Nerium oleander* flowers exhibited anticonvulsant activity against PTZ and MES in experimental animal models. Further fractionation of this extract,

may yield antiepileptic drug(s). Also, the results further buttresses the pharmacological basis for the ethnomedicinal use of the plant as antiepileptic agent.

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