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CNS activity of *Nerium indicum* flower part

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ABSTRACT

Extracts of flowers of *N. indicum* were tested orally in albino mice at the dose level of 400 mg/kg body weight for Central nervous system activity. Significant anticonvulsant activity was seen as there was a delay in the onset of Pentylenetetrazole and Maximal electroshock induced seizures as well as decrease in the severity. Significant decrease in the locomotor activity was also observed when the extract was given orally. No mortality was seen up to the dose level of 2000 mg/Kg. These results reveal the anticonvulsant and sedative activity of the extract.

Key words: *N. indicum,* anticonvulsant, Pentylenetetrazole and Maximal electroshock induced seizures

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INTRODUCTION

Nerium indicum belongs to the family Apocynaceace. The plant is commonly known as "Kaner". This is an erect, smooth shrub 1.5 – 3 meters in height and contains a cream colored, stick, resinous juice. The leaves are mostly in whorls of 3 to 4, linear lanceolate and 10-15 centimeters long with numerous horizontal nerves. The flowers are showy sweet scented, single or double 4-5 centimeters in diameter, white, pink or red and borne on terminal inflorescences (Cymes). The fruit is cylindrical, in pairs with deep linear striations, slightly twisted and 15-20 centimeters long. The seeds are numerous and compressed with white and grayish silky hairs. A wide spectrum of biological activities has been reported with various constituents isolated from different parts of the plant. Root, bark and seeds contain cardiac glycosides that have a paralyzing action on the spinal cord. Oleandrin, a pure component from the plant has a stimulating action on the heart and also a pronounced diuretic effect. The alcoholic extract shows antibacterial activity and oil obtained from the root is used in leprosy and skin diseases [1]. The plant is recognized in folk medicine as antidote, antibacterial, antileprotic, anticancer, cardio tonic and C.N.S. depressant [2-4]. In ethno botanical literature, it is mentioned to be effective in the treatment of cancer, corns and epilepsy and also used as C.N.S. depressant but no scientific data is reported. Therefore, in the present study the flower of the plant are screened for C.N.S. depressant and anticonvulsant activities.

MATERIALS AND METHODS

Collection & identification of plant material

The flowers of *Nerium indicum* were collected from the plants grown in the herbal garden in Mandsaur in India and authenticated by Dr. Gyanendra Tiwari. A voucher specimen was deposited in the department of Pharmacognosy, B.R. Nahata college of Pharmacy, Mandsaur, Madhya Pardesh, India affiliated to RGPV, Bhopal with the voucher specimen no. BRNCP/N/003/2007.

Preparation of the extract

The collected flowers were dried; powdered and successive solvent extraction was done with Petroleum ether (60-65^{0}C), methanol and water. The solvents (Petroleum ether, methanol and water) were recovered by the distillation process. Percentage yields (% w/w) of different extracts of *N. indicum* obtained by successive solvent extraction were determined and were found to be 4.171, 38.464 and 26.268 respectively.

Animals

Approval for the use of animals for the experiment had been obtained from the International Animal Ethical Committee (IAEC).Wistar rats and Swiss albino mice of either sex obtained from the Animal House unit of the Department of Pharmacology, B.R. Nahata College of Pharmacy, Mandsaur (M.P.) were used. The animals are housed in groups of 5-6 under standard laboratory

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conditions (temp. 25±2°C, relative humidity 55±5% and lighting 08:00- 20-00 hr.) with food & water ad libitum.

EXPERIMENTAL

Acute toxicity studies

Acute oral toxicity study was performed as per the Organization of Ethical Cooperation and Development (OECD) 425 guidelines [5] .Wistar strain albino rats of either sex were used for the study. The animals were kept on fasting overnight and were provided only water, after which the extracts (Pet. Ether, Methanol, and water) were administered orally at the dose level of 2000 mg/ Kg & observed for 14 days. If no mortality was observed, then the extracts were found to be safe.

Assessment of anticonvulsant activity by Maximum electroshock (MES) induced seizures [6-8]

Animals are divided into 5 groups, each group consisting of 5 animals. Control group received 1% Tween solution whereas standard group received Phenytoin 20 mg/Kg i.p. and the remaining groups received the test drug at a dose level of 400mg/Kg. After 60 min., electroshocks (150 mA for 0.2 sec.) were applied by means of stainless steel pinna electrodes. The extensor phase of convulsion process was observed.

Assessment of anticonvulsant activity by *Pentylene tetrazole (PTZ) induced seizures* [6-8]

Mice of either sex with a body weight between 18 and 22 g have been used for this study. Group I served as the control (receiving 1 % Tween solution), group II served as standard (receiving Diazepam 4 mg/Kg i.p.), remaining groups received the test drug at a dose level of 400mg/Kg .The test compound was given orally to groups of 5 mice. After 30 min 60 mg/Kg PTZ (Pentylene tetrazole) was injected i.p. Each animal was placed into an individual plastic cage for observation lasting 1 h. Seizures and tonic-clonic convulsions have been recorded.

Assessment of loco motor activity [9]

For this study, the animals were divided into five groups, each group consisting of five animals. Group I served as control (receiving 1 % Tween solution), group II served as standard (receiving Diazepam 2.5 mg/ Kg i.p.).Remaining groups received 400 mg/Kg extract. The locomotor activity was then assessed by recording the scores after every 30 min. using actophotometer.

Statistical analysis

Data obtained from pharmacological experiments are expressed as mean ±S.E.M (Standard Error Mean). Difference between the control and the treated animals in these experiments was tested for significance using ANOVA followed by Dennett's test. All statistical analysis was

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performed with prism 4.0 (Graph pad software Inc., San Diego, CA). P< 0.05 was considered significant.

RESULTS

Acute toxicity studies depicted no mortality upto the dose level of 2000 mg/Kg body weight. So, the extracts can be considered safe for long term administration. Aqueous extract of *N. indicum* at the dose level of 400 mg/Kg exhibited significant (P< 0.05) decrease in the locomotor activity as compared to control group of animals (Table 1).

Table 1: C.N.S. depressant activity of different extracts of N. *indicum*

S.No.		Locomotor recorded				
		0 min.	30 min.	60 min.	90 min.	120 min.
1.	Control	147.80±5.45	149.00±5.16	145.60±6.29	146.6±5.93	148.8±6.56
2.	Standard	150.20±3.54	119.00±2.55**	81.40±2.06**	68.00±1.73**	58.60±1.56**
3.	Pet. ether	150.60±4.20	126.80±3.77*	126.80±2.79*	90.80±2.69*	75.40±2.13*
4.	Methanol	147.60±4.20	123.20±2.47**	106.00±2.58**	86.40±3.31**	75.40±2.20**
5.	Aqueous	147.60±2.54	126.60±4.22**	98.00±1.37**	78.20±2.08**	66.40±3.42**

Values are expressed in mean ± S.E.M., n = 5 ** Significant at p < 0.01 Vs control,
* Significant at p <0.05 Vs control Dunnet's test, dose of extracts = 400 mg/kg
Standard (Diazepam) = 2.5 mg/kg

In PTZ induced seizure test, the onset of convulsions in control group was observed at 130.6 seconds after PTZ injection. The test drug delayed the onset of convulsions to 168.6 seconds (*N. indicum*, aqueous extract) (Table 2).

Table 2: Anticonvulsant activity of different extracts of *N. indicum* by P.T.Z. induced seizures

S.No.	Treatment	Onset of convulsion(sec.)	Protected animals (%)
1	Control	130.6 ± 4.02	20
2	Standard	187.0 ± .56**	100
3	Pet.ether	142.6 ± 3.10	20
4	Methanol	139.0 ± 3.36	60
5	Aqueous	168.6 ± 3.02**	80

Values are expressed in mean ± S.E.M., n = 5 ** Significant at p < 0.01 Vs control,
* Significant at p <0.05 Vs control Dunnet's test, dose of extracts = 400 mg/kg
Standard (Diazepam) = 4 mg/kg

In supra maximal electroshock seizure test, it was observed that the test drug (*N. indicum,* aqueous extract) produced a significant reduction in the duration of extensor phase which was reduced to 8 seconds (P< 0.05), while in control group this duration was observed to be 16.20seconds (Table 3).

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Table 3: Anticonvulsant activity of different extracts of *N. indicum* by M.E.S. induced seizures

S.No.	Treatment	Duration of tonic hind limb extension(sec.)	Incidence of convulsions in no. of mice
1	Control	16.20±0.86	2/5
2	Standard	9.60±0.50**	5/5
3	Pet. Ether	12.20±0.86*	2/5
4	Methanol	12.20±1.85*	2/5
5	Aqueous	8.00±0.83**	3/5

Values are expressed in mean ± S.E.M., n = 5 ** Significant at p < 0.01 Vs control,
* Significant at p <0.05 Vs control Dunnet's test, dose of extracts = 400 mg/kg
Standard (Phenytoin) = 20 mg/kg

DISCUSSION AND CONCLUSION

Acute toxicity studies indicate that the aqueous extract (flower part) of *N. indicum* may be safely used in the animals upto the dose level of 2000 mg/ Kg body weight. Decrease in the locomotor activity reveals depression effect on CNS. In the present study, the extracts of *N. indicum* significantly decreased the spontaneous locomotor activity in mice indicating central depressant effect. Saponins are known to have antagonistic activity against amphetamine, sedative property & spontaneous motor activity in experimental animals. So, the present study support to the traditional use of *N. indicum* as CNS depressant. Aqueous extract of *N. indicum* significantly inhibited the PTZ and MES induced convulsions. So, the above studies indicate that the extract of *N. indicum* possess anticonvulsant and CNS depressant activity.

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