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Research Article

STUDY OF CNS ACTIVITY OF LEAF EXTRACTS OF *NERIUM OLEANDER* IN EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

The plant *Nerium oleander* was found to use by different traditional systems for the treatment of various disorders. The aim of the present study is to investigate the effect of the different extracts of leaves of *Nerium oleander* on central nervous system (CNS) in rat and mouse. The CNS effects were evaluated by general behaviour, exploratory behaviour, muscle relaxant activity and phenobarbitone sodium–induced sleeping time using standard procedures in experimental animal models. The results revealed that the different extracts at 100 and 200 mg/kg dose levels, caused a significant reduction in the spontaneous activity (general behavioural profile), exploratory behavioural pattern (head dip test), muscle relaxant activity (rotarod), and significantly potentiated phenobarbitone sodium–induced sleeping time. The results conclude that the extract exhibit CNS depressant activity in tested animal models.

Keywords: *Nerium oleander*, Anxiolytic, Exploratory behaviour, Motor-coordination

INTRODUCTION

World Health Organization data suggest that neurological and psychiatric disorders are an important and growing cause of morbidity (presently 450 million people)[1]. More than 25% of people are affected by mental and behavioural disorders at some point during their lives. In 2000, neuropsychiatric disorders accounted for 12% of the total disability-adjusted life years (DALYs) due to all diseases and injuries, and this is projected to increase to 15% by the year 2020 as per Selye's hypothesis [2, 3]. Anxiety affects one-eighth of total population of the world and become a very important area of research interest in psychopharmacology during this decade[4]. Medical plants and plant products are the oldest and tried health-care products. Their importance is growing not only in developing countries but in many developed countries. The allopathic drugs are good in onset and have good therapeutic activity but side effects associated are poignant. Thus, the herbal medicines from natural sources with least or no side effect having similar or better therapeutic activity are best. The herbal medicines have wide therapeutic actions and safety profile. This makes the herbal therapies to be successful. One of these can be the use of *Nerium indicum* (Family: Apocyanaceae) also known as kanner is an important shrub used against various disorders in indigenous system of medicine. The shrub commonly grows. The flowers are hermaphrodite[5]. Leaves are powerful repellent. A decoction of the leaves has been applied externally in the treatment of scabies and to reduce swellings. The leaves and the flowers are cardiotonic, diaphoretic, diuretic, emetic, expectorant. It has also being reported to have antibacterial and anti diabetic activities[6, 7]. The *Nerium indicum* is an evergreen shrub growing to 4m by 4m. It is in leaf all year, in flower from June to October. The flowers are hermaphrodite [5]. The major components of it are oleander, neriin and oleandrin. The bark contains toxic glucosides, rosaginin and nerrin, volatile oil, fixed oil, etc [8]. In ancient ayurvedic literature, the plant is reported; to cure conditions of various skin disorders, wound, vomiting, burning sensation, wandering of the mind, cough. The objective of present study is to investigate the effect of petroleum ether, ethanol and aqueous extracts of the leaves of *Nerium indicum* on CNS activities in different experimental models.

MATERIALS AND METHODS

Plant material and preparation of extracts

Fresh plant of *Nerium Oleander* was collected from the rural belt of Anandapur, Odisha, during Feb-2010. It was cleaned and dried at room temperature in shade and away from direct sunlight. The plant was authenticated in the Department of Biosciences, Sardar Patel University, Anand, Gujarat. The plant was collected in bulk and washed with tap water to remove adhering soil and dirt particles and then shade dried. A voucher specimen was deposited at the school of pharmaceutical science, SOA University, Bhubaneswar. The dried plant materials were coarsely powered and stored in airtight, non-toxic polyethylene bags until used. Powdered leaves of the plant were extracted successively using soxhlet extractor with petroleum ether (60-80°C), alcohol and water as solvent for 48 h each. The three extracts petroleum ether (PEE), alcohol (ALE) and aqueous (AQE) so obtained were dried using rota-evaporator and used for further studies. The extract was suspended in distilled water, using Tween 40, as suspending agent and administered to the animals in appropriate dose levels by oral route of administration.

Animals

Swiss albino mice and wistar albino rats of either sex weighing between 25-35g and 150-220g respectively obtained from the Animal house of School of Pharmaceutical Sciences, Bhubaneswar, Odisha were used. All animals were housed in groups of six under standard environmental conditions: $25^0 \pm 2^0$ C, 45-55% Relative Humidity and 12:12 hr light/dark cycle [9, 10]. All the animals were free access of food and water *ad libitum* under strict hygienic conditions. After obtaining permission from Institutional Animal Ethics Committee (IAEC) of School of Pharmaceutical Sciences, Bhubaneswar, animal studies were performed as per rules and regulations and in accordance to the guidelines of CPCSEA. All experiments were carried out during the light period (09.00:17.00 hours).

Phytochemical characterization

The different extracts were subjected to general phytochemical analysis for presence of carbohydrates, proteins, amino acids, tannins, phenolics, flavonoids, alkaloids, anthraquinones, glycosides, saponin and steroidal nucleus using the standard methods[11, 12].

Drugs and Chemicals

Chlorpromazine hydrochloride (Indus Pharmaceuticals Limited, India), diazepam (Ranbaxy Laboratories Ltd. India), Phenobarbitone sodium (Rhone-Poulenc India Limited, India), Diethyl ether and all other chemicals of highest available purity were obtained from Merck, Mumbai, India.

Acute toxicity studies (LD_{50})

The acute oral toxicity study was carried out as per guidelines set by Organisation for Economic Cooperation and Development

(OECD)[13,14]. The median lethal dose of the pet-ether alcohol and aqueous were determined by orally administering the extracts in increasing dose levels of 0.1,0.2,0.5, 1, 1.5 and 2 g/kg body weight to healthy adult albino mice of either sex. The animals will be observed continuously for 2 h under the following profiles:

I. Behavioural profile: Alertness, restlessness, irritability and fearfulness.

II. Neurological profile: Spontaneous activity, reactivity, touches response, pain response and gait.

III. Autonomic profile: Defecation and urination.

After a period of 24 h they will be observed for any lethality or death (% of mortality).

Animal grouping and treatment

For the following activities the animals divided into eleven groups, each group containing six animals except general behavioural study. Group I for control, group II for standard, group III, IV and V for PEE (50,100 and 200mg/kg), group VI, VII and VIII for ALE (50,100 and 200 mg/kg), group IX, X and XI for AQE (50,100 and 200 mg/kg) respectively.

General behavioural profile

Evaluation of general behaviour profiles was performed by the method of Dixit and Verma et al (1976)[15], in this method albino male mice were divided into eleven groups (n=8). The PEE, ALE and AQE extract each (50,100 and 200 mg/kg,) were administered to groups III -XI, where as Groups I and II were treated with solvent (10ml/kg) and diazepam (4 mg/kg) and respectively in a similar manner. The animals were under observation for their behavioural changes, if any, at 30 min intervals in the first one hour and at 1hr intervals for the next 4hr for the following parameters.

Awareness, alertness and spontaneous activity

The awareness and alertness was recorded by visual measure of the animals' response when placed in a different position and its ability to orient itself without bumps or falls The normal behaviour at resting position was scored as (-), little activity (+), moderate flexibility (+ +), strong response (+ + +) and abnormal restlessness as (+ + + +).These responses were tested by placing the animal in a ball jar. It usually shows a moderate degree of inquisitive behaviour.

Spontaneous Activity-Moderate activity was scores as (+ +) and strong activity as (+ + +). If there is little motion, the score was (+), while if the animal sleeps, the score was (–). Excessive or very strong inquisitive activity like constant walking or running was scores as (+ + + +). A similar test was performed with the same scoring, when the animals are removed from the jar and placed on a table[16].

Touch response- This was noted when the mice were touched with a pencil or forceps (i.e. on the side of the neck, abdomen and groin)[16].

Sound response-Albino mice normally utter no sound; vocalization may indicate a noxious stimulus.

Pain response-This was graded when a small artery clamp was attached to the base of the tail.

Assessment of Phenobarbitone sleeping time

Healthy albino mice weighing between 20-30gms were fasted for 24 hrs before the experiment and were divided into eleven groups of 6 animals each. Group I was maintained as normal control which was given solvent (10 ml/kg, P.O.), group II was treated with standard drug chlorpromazine (CPZ) (5 mg/kg, P.O.), group III, IV and V were treated with different doses of ALE (50,100 and 200 mg/kg, P.O.), group VI, VII and VIII were treated with AQE (50, 100 and 200 mg/kg, P.O.) and group IX, X, XI were treated with PEE (50, 100 and 200 mg/kg, P.O.) in a similar manner. After 30minutes of administration of test drugs, Phenobarbitone sodium was administered intra-peritoneal to all groups of animals at a dose of 40mg/kg. The time between the loss and recovery of the righting reflex was taken as the sleeping time [17].

Assessment of locomotor activity

The spontaneous locomotor activity of each mouse was recorded individually for 10 min using actophotometer, which enables movement of the animal across a light beam to be recorded as a locomotion count. This test can demonstrate a CNS depressant or stimulant activity profile. The animals were allowed to adapt to the new environment for at least 5 min and then the locomotor activity was counted. The plant extract (50,100 and 200 mg/kg, P.O.) or the standard drug Diazepam (4 mg/kg P.O.) was administered 30 min before the assessment of locomotor activity. Counts were then taken after 30, 60, 90 and 120 min[18,19].

Assessment of Exploratory behaviour (Head dip test & Y-maze test)

Exploratory behaviour of the animals was evaluated using Y-maze and head dip tests.

Head dip test

Eleven groups of albino mice (n=6) were placed on the top of a wooden box with 16evenly spaced holes, 30 min after received of the extract (50, 100 and 200 mg/kg P.O., vehicle (5ml/kg,) and diazepam (4mg/kg) respectively. The number of times that each animal dipped the head into the hole was counted for a period of 3 min [20].

Y-maze test

The test was performed in different groups of 6 albino rats at 30, 60, 90 and 120 min after treatment of either Normal saline (10ml/kg), extracts (50,100 and 200 mg/kg) and diazepam(10 mg/kg) respectively. The rats were placed individually in a symmetrical Y-shaped runway (33 × 38 × 13cm) for 3 min and the number of times a rat entered in the arm of the maze with all 4ft (an 'entry') were counted [21].

Assessment of skeletal muscle relaxant activity (Motor Coordination)

Motor coordination of the mice was evaluated by using a rotarod apparatus consisting of a bar with a diameter of 3.0 cm, subdivided into five compartments by a disk 24 cm in diameter. Mice were placed on a horizontal steel rod (32mm diameter) rotating at the speed of 25 rpm. The mice capable of remaining on the top for 3 min or more, in three successive trails were selected for the study. The selected animals were divided into eleven groups (n=6). Groups were received the extracts at 50, 100 and 200mg/kg, orally, solvent (10ml/kg) and diazepam (4 mg/kg) respectively in a similar manner as above. Each group of animals was then placed on the rod at an interval of 30, 60, 90 and 120 min. The difference in the fall off time from the rotating rod between the control and the treated mice (standard-Diazepam/extract) was taken as an index of muscle relaxation [22, 23].

Statistical Analysis

Results are presented as mean ± S.E.M. Statistical significance between the groups was analyzed by means of an analysis of variance followed by Dennett's multiple comparison tests. *P* values less than 0.05 were considered significant.

RESULTS

Phytochemical Screening

The preliminary phytochemical analysis of the leaf extracts of *N.oleander* showed the presence of carbohydrates, phenols, saponins, tannins and alkaloids but devoid of steroids. All the extracts were stored in a clean glass bottles for further pharmacological studies.

Toxicity study

A preliminary acute oral toxicity study, the plant extracts produced death at dose 2000mg/kg and did not cause any death up to dose of 1500 mg/kg in mice.

Effect on behavioural profiles

The results obtained from the experiments are presented in (Table 1). The extracts affected spontaneous activity, sound and touch responses at a dose of 100 and 200mg/kg and produced moderate

or slight depression relating to awareness and alertness. However, the standard drug chlorpromazine hydrochloride caused significant depression of all these responses compared with ethanol extract. The results indicate that the extract influences general behavioural profiles, as evidence in the spontaneous activity, touch, sound and pain responses.

Table 1: Effect of extracts of *Nerium oleander* on general behavioural profile in mice

Group No	Treatment	Dose (mg/kg)	Spontaneous activity	Alertness	Awareness	Sound response	Touch response	Pain response
I	Saline	10 (ml/kg)	-	-	-	-	-	-
II	Chlorpromazine	5	++++	++	+	+++	+++	++++
III	PEE	50	++	+	+	+	++	+
IV	PEE	100	+++	++	++	+++	++	+++
V	PEE	200	++++	+++	+	+++	+++	++++
VI	ALE	50	+	++	+	++	++	++
VII	ALE	100	+++	++	++	+++	+++	++
VIII	ALE	200	++++	++	++	+++	+++	+++
V	AQE	50	++	+	++	+++	++++	++++
VI	AQE	100	+++	++	++	+++	++	++
VI	AQE	200	+++	++	++	+++	+++	+++

- No effect, + Slight depression, ++ moderate depression, +++ Strong depression, ++++ Very strong depression, n= 08

Effect on phenobarbitone sodium-induced sleeping time

The test extracts except 50mg/kg, significantly potentiated the Phenobarbitone sodium-induced sleeping time, with respect to the control (Table 2). The duration of sleeping time in phenobarbitone induced experimental model increases in a dose dependent manner. The Potentiation of phenobarbitone sodium-induced sleeping time is possibly through a CNS depressant action or a tranquilizing action.

Effect on Locomotor activity on mice

A significant decrease in locomotor activity was observed in case of petroleum ether, aqueous and ethanolic extracts at different doses High dose of ALE, PEE and AQE extracts (100 and 200 mg/kg p.o) and diazepam (4 mg/kg P.O.) decreased the locomotor activity significantly (P<0.01) whereas, low dose of (50 mg/kg P.O.) did not show a significant reduction in the locomotor activity (Table 3).

Table 2: Effect of various extracts of *Nerium oleander* at different dose levels on Phenobarbitone sodium induced sleeping time

Groups	Treatment	Dose(mg/kg)	Sleeping time (min.)
I	Solvent	10ml/kg	33 ± 1.58
II	Chlorpromazine	5	64.66 ± 7.9**
III	PEE	50	35.16 ± 2.7
IV	PEE	100	58.6 ± 3.1*
V	PEE	200	90.8 ± 4.01**
VI	ALE	50	36.5 ± 1.78
VII	ALE	100	72 ± 7.3**
VIII	ALE	200	103 ± 3.5**
IX	AQE	50	33.6 ±1.97
X	AQE	100	83.3 ± 3.9**
XI	AQE	200	95.6 ± 6.4**

One way ANOVA Followed by Dunnet's Test. Values are expressed as Mean ± SEM; n=6 in each group; Statistical significance denoted as *P<0.05 and **P<0.01 when compared to control.

Table 3: Effect of different extracts of *N.Oleander* on locomotor activity in mice

Groups	Treatment	Dose (mg/kg)	Locomotor activity observed during10min	
			Before	After
I	Solvent	10ml/kg	222.33 ± 7.36	221.33 ± 6.94
II	Diazepam	4	215.6 ± 13.28	143.66 ± 10.16**
III	PEE	50	202.83 ± 13.15	199 ± 14.47
IV	PEE	100	211.83 ± 7.09	162.16 ± 9*
V	PEE	200	218.16 ± 10.4	120 ± 6.29**
VI	ALE	50	200.5 ± 7.85	195 ± 10.55
VII	ALE	100	213 ± 10.66	152 ± 7.06**
VIII	ALE	200	216 ± 8.53	114.16 ± 4.61**
IX	AQE	50	215.33 ± 8.07	211.33 ± 7.31
X	AQE	100	22.5 ± 6.05	143.66 ± 7.71**
XI	AQE	200	208.5 ± 8.83	106 ± 8.32**

Values are expressed as Meann ± SEM, n=6 and analysed by one-way ANOVA followed by Dunnet's Test; *P<0.05 and **P<0.01 when compared to control.

Effect of extracts on exploratory behavioural potential

Head dip test

The head dip test revealed that ALE, PEE and AQE at 200 mg/kg dose level significantly reduced the number of head dips on a wooden board with 16 evenly spaced holes within 3 minutes, while standard drug diazepam also showed a significant reduction in the head dips responses occurred in mice treated with the extracts compared with the control (Table 4).

Y-Maze Model

A significant decrease in the number of visits in the arms of the Y-maze was observed in the Diazepam treated animals as compared to the control animals. Both the doses (100 and 200 mg/kg) of *N. oleander*

showed a significant decrease in the number of visits in the arms of the Y-maze which was comparable with the standard Diazepam (Table 5).

Effect on Motor co-ordination on mice

The result from the rotarod test showed (Table 6) that the extracts significantly reduced the motor co-ordination of the tested animals. This test, (100 and 200 mg/kg, P.O.) significantly reduced the time spent by the animals on revolving rod when compared to control (P<0.01). The standard drug (diazepam) also showed significant effect when compared to control (P<0.01) Low dose of drug (50 mg/kg) did not show any significant effect.

Table 4: Effect of different extracts of *Nerium oleander* on exploratory behaviour (Head dip test) in mice

Groups	Treatment	Dose(mg/kg P.O. route)	Head dip test
I	Solvent	10ml/kg	99.16 ± 2.66
II	Chlorpromazine	5	29 ± 2.36
III	PEE	50	95.83 ± 3.36
IV	PEE	100	37.5 ± 1.6
V	PEE	200	25.6 ± 3.35
VI	ALE	50	93.83 ± 4.79
VII	ALE	100	35.33 ± 2.7
VIII	ALE	200	27.66 ± 2.62
IX	AQE	50	93 ± 5.07
X	AQE	100	37.3 ± 2.72
XI	AQE	200	26.16 ± 3.85

Values are expressed as Mean ± SEM; n=6; analysed by One way ANOVA followed by Dunnet's t-test ; *P<0.05 and **P<0.01 when compared to control.

Table 5: Effect of *Nerium. oleander* on exploratory behaviour (Y-maze test) in rats

Groups	Treatment	Dose (Mg/kg)	Number of entries after treatment (min)			
			30	60	90	120
I	Solvent	10ml/kg	10 ± 0.73	10.5 ± 1.17	10.33 ± 1.05	10.5 ± 0.76
II	Diazepam	4	5.6 ± 0.8**	4.6 ± 0.71**	3.66 ± 0.76**	3.83 ± 0.6**
III	PEE	50	11.33 ± 0.88	12.83 ± 1.3	10 ± 0.57	11.5 ± 1.17
IV	PEE	100	6.66 ± 0.88*	7.66 ± 1.02*	6.33 ± 0.76*	7.5 ± 0.92*
V	PEE	200	4.83 ± 0.83**	4.33 ± 0.61**	3.83 ± 0.6**	3.33 ± 0.49**
VI	ALE	50	10.66 ± 1.2	9.66 ± 1.38	10 ± 0.57	10.33 ± 0.95
VII	ALE	100	6.16 ± 0.87*	5.16 ± 0.83**	4.33 ± 0.66**	4.33 ± 0.84**
VIII	ALE	200	5.66 ± 0.57**	4.16 ± 0.6**	4.16 ± 0.17**	4 ± 0.77**
IX	AQE	50	8 ± 0.57	7.83 ± 0.94	8.33 ± 0.76	8 ± 0.57
X	AQE	100	5 ± 0.85**	4.16 ± 0.4**	3.66 ± 0.55**	4.33 ± 0.76**
XI	AQE	200	4 ± 0.73**	3.5 ± 0.42**	3.83 ± 0.4**	4.16 ± 0.6**

Values are expressed as Mean ± SEM; n=6; analysed by One way ANOVA followed by Dunnet's t-test ; *P<0.05 and **P<0.01 when compared to control.

Table 6: Effect of different extracts of *Nerium oleander* on motor coordination in mice

Gr.	Treatment	Dose(mg/kg)	Time of fall (sec)			
			30min	60min	90min	120min
I	Solvent	10ml/kg	315.3 ± 6.27	313.1 ± 13.1	312.3 ± 13.1	308 ± 10.22
II	Diazepam	4	151.3 ± 12.3**	115.8 ± 5.66**	73.66 ± 3.56**	64.16 ± 4.77**
III	PEE	50	313.6 ± 10.6	317.5 ± 12.07	301.8 ± 4.76	300.5 ± 7.34
IV	PEE	100	298.5 ± 14.9	261.8 ± 18.09*	248.5 ± 8.7*	246.8 ± 16.63*
V	PEE	200	156.3 ± 15.2**	118.5 ± 7.57**	89.16 ± 4.47**	80 ± 3.75**
VI	ALE	50	309.5 ± 13.82	301.6 ± 14.37	304.5 ± 4.89	292.8 ± 7.08
VII	ALE	100	271 ± 7.26*	208.8 ± 13.97	181.8 ± 13.37**	173.1 ± 13.75**
VIII	ALE	200	134.1 ± 4.3**	101.3 ± 4.2**	85.83 ± 3.9**	83.33 ± 4.04**
IX	AQE	50	304.5 ± 6.67	302.8 ± 7.91	306.8 ± 8.6	304.8 ± 4.9
X	AQE	100	289.6 ± 11.10*	237.3 ± 22.13**	203.5 ± 13.38**	180.8 ± 14.94**
XI	AQE	200	142.3 ± 8.35**	113.1 ± 5.6**	85.83 ± 3.9**	83.33 ± 4.04**

Values are expressed as Mean ± SEM; n=6; analysed by One way ANOVA followed by Dunnet's t-test ; *P<0.05 and **P<0.01 when compared to control.

DISCUSSION

In the present study different extracts (PEE, ALE, AQE) of *N. oleander* leave were studied for CNS activity using several animal models such as locomotor activity, muscle relaxant activity, exploratory behaviour and Phenobarbital induced sleeping time. The preliminary phytochemical analysis of the extracts of *N. oleander*, revealed the presence of carbohydrates, tannins, flavonoids and saponin. The results indicate that, the ALE, PEE and AQE influences general behavioural profiles, as evidenced by decrease in the alertness, reactivity to touch and auditory stimuli indicative of depressant profile. The PEE, ALE and AQE significantly dose-independently reduced the onset and prolonged the duration of sleep induced by phenobarbitone. By potentiating the phenobarbitone -induced sleep, the extracts seems to possess sleep inducing properties [24].potentiated the phenobarbitone induced sleeping time in a dose dependent manner, possibly through CNS depressant action or tranquilizing action[25, 26]. Locomotor activity is considered as an index of alertness and a decrease in it is indicative of sedative activity [27].

N. oleander extracts decreased locomotor activity at all the tested doses reinforcing the CNS depressant effect. Sedative hypnotic agents act to increase gamma amino butyric acid (GABA) mediated synaptic inhibition either by directly activating GABA receptors or, more usually, by enhancing the action of GABA on GABAA receptors.

Benzodiazepines and barbiturates are examples of widely used therapeutic agents that act as positive allosteric modulators at GABAA receptors [28].The ability of the extract to potentiate the sedative property of diazepam suggests that it may possibly act by interacting with GABA-mediated synaptic transmission. The reduction in exploratory behavioural study pertaining to head dip revealed the CNS activity of the test extracts. The possible CNS activity of the ethanolic, aqueous and petroleum ether extract was further tested against other common psychological tests (i.e. the rotarod test). A significant lack in motor coordination and muscle relaxant activity were noted in animals treated with the test extracts. The results of the present studies reveal that, the ALE, AQE and PEE may have CNS depressant activity like psychopharmacological agents. The drug(s) showing positive response in gross behavioural parameters, muscle in-coordination, exploratory behaviour must have effect on the region of brain[29, 30]. It may be suggested that the extracts have effect on the region of brain, which is responsible for the behavioural and other tested parameters.

CONCLUSION

Based on the results of the present study of different extracts on psychopharmacological tests, we conclude that the extracts at 100 and 200mg/kg possess strong CNS depressant activity. Aqueous and alcoholic extracts showed the CNS depressant activity in dose dependent manner. The reduction in exploratory behaviour in animals is similar with the action of other CNS depressant agents. A significant lack in motor coordination and muscle relaxant activity was also noted in animals treated with crude extract. The results altogether indicates that the extract shows CNS depressant activity. However, further studies are necessary to examine underlying mechanisms of CNS depressant effects and to isolate the active compound (s) responsible for these pharmacological activities.

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