

ANTI-ANXIETY ACTIVITY STUDIES OF VARIOUS EXTRACTS OF *NERIUM OLEANDER* LINN. FLOWERS

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ABSTRACT

The clinical applications of well-known benzodiazepines as anxiolytic agents are limited because of their side effects. Therefore, the development of new pharmacological agents, from medicinal plants is well justified. The purpose of this study was to explore the anti-anxiety effects of the various extracts of *N. oleander* flowers. Petroleum ether, chloroform, ethyl acetate, and methanol extracts of *N. oleander* flowers were prepared by successive extractions using a Soxhlet apparatus, and subsequently evaluated for anti-anxiety activity using the EPM model. Diazepam was used as standard drug. In chloroform and ethyl acetate extract of *N. oleander* flowers showed significant increases in open arm entries and mean time spent in open arms at the dosages of (25 and 50 mg/kg) and (100 and 200mg/kg) respectively. The most pronounced effect of chloroform and ethyl acetate extract is produced at the dose of 25 and 200 mg/kg respectively. It was concluded that the chloroform and ethyl acetate extract showed anxiolytic effect on mice and it could serve as a new approach for the treatment of anxiety.

Keywords: Anti-anxiety, Elevated plus maze, *Nerium oleander*.

INTRODUCTION

The importance, necessity and potentiality of medicinal plants in the practice of medicine today are well established and cannot be underestimated. The use of herbal preparation for therapeutics remedies is as old as man himself. *N. oleander* is an important medicinal plant of family Apocynaceae commonly known as Kaner is large glabrous evergreen shrub with milky juice. This plant grows in Mediterranean region up to Iran and India. Leaves are three, shortly stalked, coriaceous, 10-15 cm long, linear lanceolate with dark green colour. Flowers are salver-shaped pink or white scentless without any fragrance¹. From the genus *Nerium*, a number of derivative inaccessible metabolites have been reported. Triterpenes, pregnanes, cardenolides, cardiac glycoside were isolated and characterized². A number of pharmacological activities have been reported such as cardiotoxic³, diuretic⁴, cytotoxic⁵, antibacterial⁶, anti inflammatory⁷, antifungal⁸, depressant action on central nervous system (CNS)⁹ and antitumor¹⁰. There are records that the root is bitter; aphrodisiac, very poisonous, but an antidote to snake venom. It can also be used as good tonic for chronic pain either in abdomen or in joints. For chancres and ulcers on the penis, it is recommended to apply with the root beaten into a paste with water. The fluid of young leaves is poured into eyes in ophthalmia with copious lachrymation. Moreover, Vydians consider that the root bark and the leaves of this shrub, if applied externally¹¹ are very powerful repellents. The plant can also be used as a rodenticide, insecticide and for curing indigestion, fever, ringworm, leprosy, venereal disease¹². The clinical applications of well-known benzodiazepines as anxiolytic agents are limited because of their side effects. Therefore, the purpose of the investigation was to test anti-anxiety property of *N. oleander* flower's extract as new pharmacological agents.

MATERIALS AND METHODS

Plant material and preparation of extracts

The *N. oleander* flowers were collected from Kota district of Rajasthan in India and authenticated by routine pharmacognostic procedures by Dr. S. N. Sharma, senior scientist, botany division of Indian Institute of Integrative Medicine (IIIM), Jammu, India. A voucher specimen was retained and deposited at the crude drug repository of the herbarium of IIIM, vide CDR accession No. 21869.

The flowers of *N. oleander* were dried in shade and coarsely powdered. 1kg flowers were successively extracted in the soxhlet apparatus with petroleum ether, chloroform, ethyl acetate, methanol and water for the complete extraction of the phytochemicals. The five extract thus obtained were dried under rota evaporator at 45 °C

(Heidolph) and the dried extracts were stored in vacuum desiccators containing anhydrous silica gel.

Preliminary phytochemical screening

The plant material and the extract were screened for the presence of various chemical constituents (alkaloid, tannins, cardiac glycoside, steroids, terpenoids, flavonoids, anthraquinones, reducing sugar, anthocyanoside and saponins) using standard procedures¹³.

Animals

Male Swiss albino mice (8-12 weeks old) weighing 18-20 g were purchased from Central Animal House Facility, IMTECH, Chandigarh, 55/1999/CPCSEA. The protocol for CNS studies was approved by the Institution Animal Ethics Committee (IAEC) of ASBASJSM College of Pharmacy, Bela (Ropar) Punjab (Registration No. 724/02/a/CPCSEA-29/10/2002). The animals were housed under standard laboratory conditions with temperature (23 ± 1°C), relative humidity (55 ± 10%), 12/12 h light/dark cycles and fed with standard pellet diet (M/s Ashirwad Industries, Mohali) and purified water *ad libitum*.

Drugs preparation

Test doses of various fractions were freshly prepared as a homogenized suspension of *N. oleander* flower's extract in doses of 100, 200 and 400 mg/kg except chloroform extract (25, 50 and 100 mg/kg) administered orally to Swiss albino mice once, during the experiment. Diazepam used as standard.

Acute toxicity study

The maximum tolerable dose determination was performed using OECD (Organization for Economic Corporation and Development) guideline 423. The study was performed as a stepwise procedure to explore toxicity at dose level 5, 50, 300 and 2000 mg/kg, p.o. with 3 animals, at each dose level. Three female Swiss mice (18-22 g) were used for the study and were not fed for 3-4 h prior to the experiment. They were individually administered various extract of *N. oleander* (2000 mg/kg, p.o.) on day 1st of the experiment. Each animal was continuously monitored during first 30 min, and then they were monitored on an hourly basis for next 4 hours. Subsequently, they were observed after a four hour interval. After this, they were under observation for 14 days to monitor any abnormal signs and symptoms depicting toxicity. These animals were humanely killed for animal welfare reasons after termination of the experiment. The animals were observed for any change in skin and fur, respiratory, circulatory, autonomic and central nervous systems, somatosensory activity and behavioral pattern. Attention was given to observation

like tremors, convulsions, salivation, diarrhoea, lethargy, sedation, hypnosis and coma¹⁴.

Spontaneous motor activity

The animals were divided into 14 groups, with each group constituting 6 mice. For spontaneous motor activity, every mice was introduced into the actophotometer (INCO) and its score of locomotor was measured for 10 min duration¹⁵.

Group-1	Control	
Group-2	Standard (Diazepam)	(Dia.) 4 mg/kg
Group-(3-5)	Petroleum ether extract	(PE) (100, 200 and 400 mg/kg p.o.)
Group-(6-8):	Chloroform extract	(CE) (25, 50 and 100 mg/kg p.o.)
Group-(9-11)	Ethyl Acetate extract	(EA) (100, 200 and 400 mg/kg p.o.)
Group-(12-14)	Methanol extract	(ME) (100, 200 and 400 mg/kg p.o.)

Elevated plus maze test (EPM)

To conduct the EPM test 48 mice were taken and divided into 8 groups with each group consisting 6 mice. The EPM consists of two open arms and two closed arms (16 x 05 x 25 cm each) elevated to a height of 25 cm. 1 h post treatment, each mouse was placed in turn in the centre of the maze facing one of the open arms. The cumulative times spent by each mouse in the open and closed arms of the maze were recorded for 5 min¹⁶.

Group-1	:	Control
Group-2	:	Dia. 2 mg/kg
Group-(3-5):	:	CE (25, 50 and 100 mg/kg p.o.)
Group-(6-8):	:	EA (100, 200 and 400 mg/kg p.o.)

RESULTS

Phytochemical screening

Results obtained in this exercise revealed the presence of alkaloid, saponins, sterols carbohydrates, tannins, flavanoids, fatty acid, terpenoid and glycosides.

Table1: Phytochemical constituents present in various extracts of *N. oleander* flowers

S. No.	Constituents	PE	CE	EA	ME
1	Alkaloids	-	√	√	√
2	Saponins	-	-	-	√
3	Sterols	√	√	-	-
4	Carbohydrates	-	√	√	√
5	Tannins	-	-	√	√
6	Flavanoids	-	-	√	√
7	Fatty acid	√	-	-	-
8	Terpenoid	-	-	√	-
9	Glycosides	√	√	√	√

Spontaneous motor activity

In this study all extract were administrated orally at three selected dose level at 0 h (Fig.1). Among all these extract, EA and CE were produced significant reduction ($P < 0.01$) in locomotor activity after 1 h of oral administration (Fig. 2) at the dose level (200 and 400 mg/kg) and 100 mg/kg respectively. The lower dose of EA and CE is ineffective in bringing a significant change in the observed activities. However, after 2 h of oral administration (Fig. 3) of EA (200 and 400 mg/kg) and CE (50 and 100 mg/kg) caused highly significant reduction ($P < 0.001$) in all activities observed in comparison to control.

Elevated plus maze test

EA and CE of flower at doses of (100 and 200 mg/kg) and (25 and 50 mg/kg) respectively have shown the highly significant ($P < 0.001$) in the cumulative time spent in the open arms in comparison to the control. The most pronounced effect of EA and CE was produced at the dose of 200 and 25 mg/kg respectively. This effect was greater but not significantly different from the effects those produced by diazepam. At the higher doses of 100 mg/kg (CE) and 400 mg/kg (EA), the *N. oleander* has produced no significant increase in the time spent in the open arm.

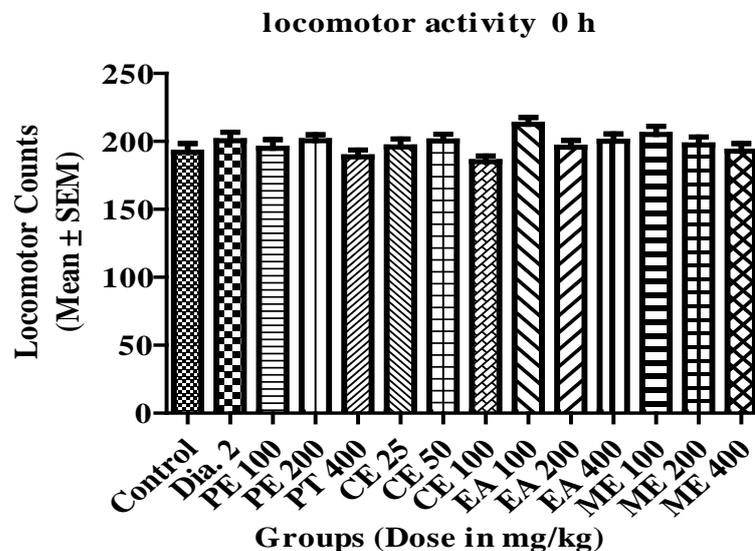


Fig. 1: Effect of following successive solvent extracts of *N. oleander* flowers on locomotor activity at 0 h after oral administration in mice

One way ANOVA followed by Dunnett's test; No. of animals per group = 6.

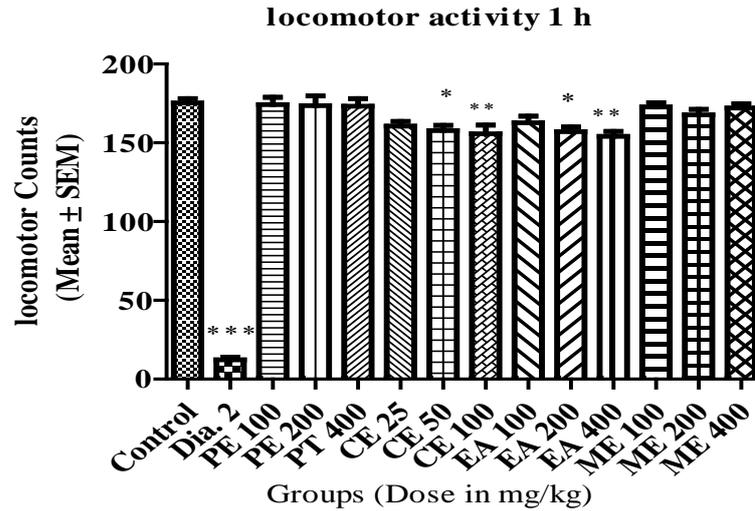


Fig. 2: Effect of following successive solvent extracts of *N. oleander* flowers on locomotor activity at 1h after oral administration in mice. One way ANOVA followed by Dunnett's test; *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ vs. vehicle treated control group; No. of animals per group = 6.

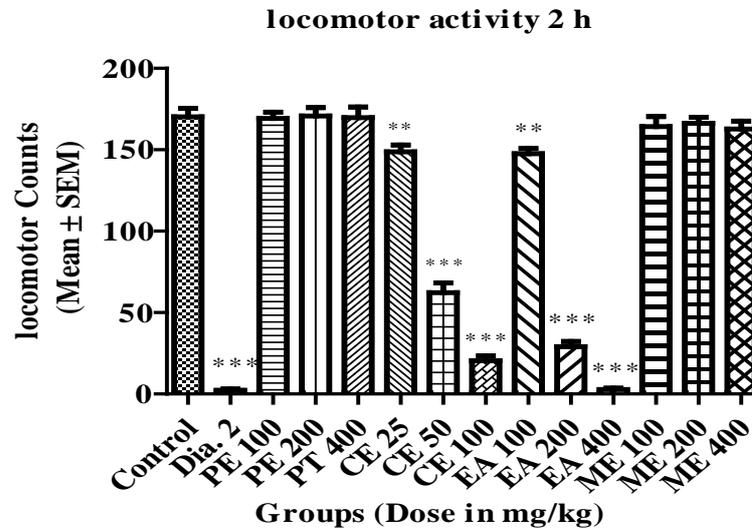


Fig. 3: Effect of following successive solvent extracts of *N. oleander* flowers on locomotor activity at 2h after oral administration in mice. One way ANOVA followed by Dunnett's test; *** $P < 0.001$, ** $P < 0.01$, vs. vehicle treated control group; No. of animals per group - 6.

Table 2: Effect of various extracts of *N. oleander* flowers on Anti-anxiety activity in mice

Treatment	Dose (mg/kg) p.o	Mean number of entries in open arm (Mean ± SEM)	mean time spent in open arm (seconds) (Mean ± SEM)
Control	-	0.83 ± 0.4	3.3 ± 0.42
Dia.	2	8.5 ± 1.1***	19 ± 1.4***
CE	25	4.7 ± 0.67***	11 ± 1***
	50	2.5 ± 0.43**	4.7 ± 0.56**
	100	2.3 ± 0.49	3.8 ± 0.6
EA	100	1.7 ± 0.33***	10 ± 0.79***
	200	5.7 ± 0.49***	16 ± 1.4***
	400	3.0 ± 0.37	5.2 ± 0.48

Values expressed are mean ± SEM from 6 rats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared to control group. One way ANOVA followed by Dunnett's test.

DISCUSSION

Maximum safe dose of various extracts of *N. oleander* flowers was obtained by acute oral toxicity study followed by OECD guideline 423, such as oral administration of PE, EA, ME and water extract were safe at 2000 mg/kg body weight, however CE was safe at 500 mg/kg body

weight. This study explored the putative behavioural effects of the various extract of *N. oleander* in mice. The EA and CE were proficient to induce motor depressant effects after the oral administration. Thus, single doses of CE (50 and 100 mg/kg) and EA (200 and 400 mg/kg) produced a significant decrease in total motility.

Decrease in locomotion implies depression effect on CNS¹⁷ also it has been well-known that an augment in concentration of GABA may lead to CNS depressant effect¹⁸. The favourable medicinal property of plant materials evidently results from the combinations of secondary metabolites present in the plant, through additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process¹⁹. This fact reveals that medicinal activities of plants are distinctive to particular plant species or groups, dependable with the conception that combinations of secondary metabolites in a particular plant are generally taxonomically diverse²⁰. Preliminary phytochemical analysis of various extracts of *N. oleander* flowers in this study revealed the presence of alkaloids, tannins, cardiac glycosides, steroids, terpenoids, flavonoids, reducing sugars, and saponins. Going through this case, the anxiolytic and sedative activities observed with CE and EA of flowers was possibly due to the presence of flavonoids, alkaloids, and terpenoids in the plant extract. Some synthetic and natural flavonoids have been bind exclusively and competitively to benzodiazepine receptors and reveal anti-anxiety property in the EPM test in rat and mice²¹, these extracts induce anxiolytic like effect. These findings indicate a remarkable sedative effect of this plant. In fact, only mice treated with *N. oleander* 25 and 50 mg/kg (CE) and 100 and 200 mg/kg (EA) showed a significant increase of both the percentage of entries and the percentage of time spent in the open arms of the EPM. Montgomery reported that rodents consistently spend greater time in the closed arms when placed in maze comprising of open and closed arms²². Avoidance of the open arm portrays a manifestation of fear and anxiety, based on these assertions EPM test is reliable means of identifying selective anxiolytic effects of drugs. Handley and Mithani further revealed that open arm avoidance was enhanced by anxiogenic agent (picrotoxin) and reduced by anxiolytic agent (Dia.)²³. The open arm/closed arm approach for screening of anxiolytic effect has worked well in identifying the anxiolytic potential of benzodiazepine/GABA_A receptor related agents, while not being reliable in detecting anti-anxiety effects through unrelated mechanisms, e.g. 5-HT_{1A} partial agonists like buspirone²⁴. In this context, the effectiveness of *N. oleander* (50 and 100 mg/kg) in relieving anxiety in this model suggests a possible positive modulation of the GABA_A chloride channel receptor complex. At higher doses of 200 and 400 mg/kg, the anxiolytic effect of *N. oleander* was sustained but diminished in magnitude, being non-significant at the later dose. The EPM model utilized in this research is a suitable animal model as this model has invoked natural stimuli²⁵. A CE and EA significantly increased the time spent in open arms and the frequency of open arm entries in EPM, thus suggesting an anti-anxiety effect²⁶.

Conclusion: It was concluded that the CE and EA of *N. oleander* flowers showed an anxiolytic effect on mice, and it could serve as a new approach for the treatment of anxiety.

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