

EVALUATION OF SKELETAL MUSCLE RELAXANT ACTIVITY OF AQUEOUS EXTRACT OF NERIUM OLEANDER FLOWERS IN ALBINO RATS

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ABSTRACT

Background: Aim: Skeletal muscle relaxants are drugs that act peripherally at neuromuscular junction or centrally in the cerebrospinal axis to reduce muscle tone and or cause paralysis. The side effects of these skeletal muscle relaxants cause them to be used with caution *Nerium oleander* is an evergreen shrub with medicinal properties used in various disorders. One of it's use is in skeletomuscular disease since it has skeletal muscle relaxant activity. Herbal medicines have wide therapeutic actions and safety profile. This makes the herbal therapies to be successful. **Materials and Methods:** The aqueous extract of *Nerium oleander* flowers (NA) was prepared using the soxhlet apparatus. 24 Swiss albino rats aged 6-7 weeks of either sex, weighing about 150-200g were taken. The animals were divided into 4 different groups. Group 1: Normal saline 10 ml/kg (control) , Group 2:Diazepam 10 mg/kg (standard) , Group 3:NA at a dose of 200 mg/kg (study group 1) , Group 4 : NA at a dose of 400 mg/kg (study group 2) . Skeletal muscle relaxant activity was tested using Rotarod and actophotometer models. Comparison of four groups was done with ANOVA. Multiple comparisons was done with Tukey's Post Hoc. **Result:** Aqueous extract of *Nerium oleander* (NA 200mg/kg and 400mg/kg) has shown significant reduction in time spent on rotarod when compared to control group animals (p <0.001). In Actophotometer model, there was highly significant reduction in locomotor activity in both the test groups (NA 200mg/kg and 400mg/kg) when compared to control group animals (p <0.001). In both the models, Aqueous extract of *Nerium oleander* flowers showed dose dependent increase in muscle relaxation. **Conclusion:** From the above observations we can conclude that aqueous extract of *Nerium oleander* flowers showed dose dependent muscle relaxant activity at doses 200 and 400 mg/kg.

INTRODUCTION

During the 16th century, European explorers found that natives in the Amazon Basin of South America were using curare, an arrow poison that produced skeletal muscle paralysis, to kill animals.^[1] The active compound, d- tubocurarine, and its modern synthetic derivatives have had a major influence on the practice of anesthesia and surgery and have proved useful in understanding the basic mechanisms involved in neuromuscular transmission.^[2]

Skeletal muscle relaxants are drugs that act peripherally at neuromuscular junction/muscle fibre itself or centrally in the cerebrospinal axis to reduce muscle tone and/or cause paralysis.^[3]

The term "Muscle relaxant" is used to refer to two major therapeutic groups:

1. Neuromuscular blockers
2. Spasmolytics / centrally acting muscle relaxants

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The neuromuscular blocking agents are used primarily in conjunction with general anaesthetics to provide muscle relaxation for surgery.

Centrally acting muscle relaxants are used mainly for painful muscle spasms and spastic neurological conditions.^[3] The side effects of antispasmodic agents and antispasticity agents cause them to be used with caution . Previous reports have shown that 10-20% of adults suffer from insomnia. Thus looking for an effective alternative has always been a priority in this regard.^[4] The herbal medicines have wide therapeutic actions and safety profile. This makes the herbal therapies to be successful.

Nerium oleander, (Kaner in Hindi and Kanagalu in kannada) is an evergreen shrub or small tree which belongs to Apocynaceae family and all its parts are potentially toxic . It is so widely cultivated that no precise region of origin has been identified though Southwest Asia has been suggested. According to the literature survey, it is a valuable medicinal plant used

in all systems of medicine. It is a herb with different colored flowers and is suited for the dry locality.^[5]

Oleander is one of the most poisonous of commonly grown garden plants. Oleander grows to 2–6 m (6.6–19.7 ft) tall, with erect stems that splay outward as they mature. The leaves are in pairs or whorls of three, thick and leathery, dark-green, narrow lanceolate, 5–21 cm (2.0–8.3 inch) long and 1–3.5 cm (0.39–1.38 inch) broad, and with an entire margin 6. The flowers grow in clusters at the end of each branch; they are white, pink to red, 2.5–5 cm (0.98–1.97 inch) diameter with a deeply 5-lobed fringed corolla round the central corolla tube. The flowers are salver-shaped pink or white without any fragrance.^[6] The phytochemicals identified in various parts of the plant include mainly cardiotonic glycosides, terpenoids, and steroids.

The leaves and the flowers are cardiotonic, diaphoretic, diuretic, and emetic, expectorant. It has also been reported to have antibacterial and anti diabetic activities.^[7]

Previous studies have shown that the aqueous extract of the leaves of N.oleander had reduced locomotor activity in experimental animals like albino rats. In this study, we would like to elucidate whether the flowers of N. oleander plant had reduced locomotor activity as that of aqueous extract of the leaves of N. oleander.

A preliminary acute oral toxicity study, the flower extract produced no adverse effects at dose 2000 mg/kg and did not cause any death up to a dose of 3000 mg/kg in mice.^[6]

Objective: To evaluate the skeletal muscle relaxant activity of aqueous extract of Nerium oleander flowers in albino rats in comparison to diazepam.

MATERIALS AND METHODS

The study was initiated after seeking the necessary approval from the Institutional Ethics Committee, JJM Medical College in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals, India).

Plant

The pink flowers of N. Oleander were collected from their natural habitat in Davangere city Karnataka, India and authentication was done at the Dept of botany, Davangere. Flowers were dried in shade, pulverized by a mechanical grinder and passed through mesh sieve to get the coarse powder.

Preparation of extract

The aqueous extract of flowers was prepared using the soxhlet apparatus in the Department of Pharmacology. The extract was dried, stored at room temperature and protected from direct sunlight.

Selection of animals: A total of 20 Swiss albino rats aged 8–10 weeks of either sex weighing about 100–150 g were obtained from the animal house of JJM Medical college The animals were fed standard pellet diet and with water, and were maintained under

standard conditions of temperature, humidity, and light (12 h light/12 h dark cycle).

Inclusion Criteria

- Healthy 20 albino rats weighing 100-150 gm.
- The mice previously unused for any experiments.

Exclusion Criteria

- Pregnant and diseased animals were not included in the study.

Duration of study: 2 months

Drugs and chemicals: Diazepam at a dose of 10mg/kg, Normal saline at a dose 10ml/kg and Aqueous extract of Nerium oleander flowers (NA) at doses of 200mg/kg and 400mg/kg were used in this study. All drugs were given orally and administered one hour prior to testing.

Instruments: Rotarod and Actophotometer

Procedure: Albino rats of either sex, weighing around 100 – 150 gms were randomly selected. All the animals were kept under laboratory condition for acclimatization period of seven days before carrying out the experiments. Each group of mice was housed separately in a metallic cage and provided with adequate food and water. They were maintained under standard laboratory conditions with natural light and dark cycle, under room temperature. The animals were divided into four groups of five rats each.

The drugs were administered as shown below

- Group I - Control rats (normal saline 10 mL/kg)
- Group II - standard (diazepam 10 mg/kg)
- Group III - NA at a dose of 200 mg/kg
- Group IV - NA at a dose of 400 mg/kg.

Laboratory models for evaluating Skeletal muscle relaxant activity

i) Rotarod

The test is used to evaluate the activity of drugs interfering with motor coordination. In 1956, Dunham and Miya suggested that the skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod (fig 3).

Procedure:

The apparatus consists of a horizontal wooden rod or metal rod coated with rubber with 3 cm diameter attached to a motor with the speed adjusted to 2 rotations per minute. The rod is 75 cm in length and is divided into 6 sections by plastic discs, thereby allowing the simultaneous testing of 6 mice. The rod is in a height of about 50 cm above the table top in order to discourage the animals from jumping off the roller.

After the administration of control, standard, and test material, the fall off time from the rotating rod was noted after 30 min.

The difference in the fall off time from the rotating rod between the control and the treated rats was taken as an index of muscle relaxation.

ii) Photoactometer

The spontaneous locomotor activity was assessed with the help of a photoactometer. Each animal was

observed for a period of 5 min in a square closed field arena (30 cm × 30 cm × 30 cm) equipped with six photocells in the outer wall. Interruptions of photocell beams (locomotor activity) were recorded by means of a six digits counter. To see the locomotor activity, the Actophotometer was turned on and each mouse was placed individually in the activity cage for 5 min. The basal activity score for all the animals was noted. Control normal saline, standard diazepam, and two different doses of aqueous extract of Nerium oleander flowers (NA) were given orally and after 1-h of re-testing, the activity score for 5 min was observed. The difference in the activity, before and after drug administration, was noted. The percentage decrease in motor activity was (fig 4) .

Statistical Analysis: Statistical analysis was carried out with SPSS version 20 for windows. Mean and standard deviation were calculated for continuous variables.

Comparison of four groups was done with One Way Analysis of Variance (ANOVA). Multiple comparisons done with Tukey's Post Hoc.

RESULTS

Rotarod: For muscle relaxation, in this test, (NA 200 mg/kg and 400 mg/kg) showed highly significant

reduction in the time spent by the animals on the revolving rod when compared to the control ($P < 0.000$). The standard drug (diazepam) also showed a highly significant effect when compared to the control ($P < 0.000$). However, two different doses of NA (200 and 400 mg/kg p.o.) showed a dose-dependent increase in muscle relaxation, that is, $202.17 \pm 70.56^{**}$ and $110.33 \pm 50.60^{**}$, respectively, when compared to the control (Table 1 and fig.1). Maximum muscle relaxation was observed with 400 mg/kg of NA. The result from the Rotarod test showed that the alcoholic extract of Nerium oleander flowers significantly reduced the motor coordination of the tested animals.

In Actophotometer model, spontaneous locomotor activity is measured and compared between the groups. Standard drug Diazepam significantly reduced the number of crossings of light beams which is taken as locomotor activity when compared to control (401.50 ± 50) ($p < 0.001$). Animals treated with NA 400mg/kg showed greater decrease in locomotor activity (212.83 ± 37.04) than animals treated with NA 200mg/kg (258.17 ± 19.35) (table 1 and fig 2) and therefore showed a statistically significant dose-dependent anxiolytic effect of NA ($p < 0.002$) . The reduction in locomotor activity with 200mg/kg and 400mg/kg is significant when compared to standard group (159.00 ± 33.65)

Table 1: Effects of NS (GROUP I), Diazepam (GROUP II), NA : 200mg/kg(GROUP III) and 400mg/kg(GROUP IV) IN ROTAROD AND ACTOPHOTOMETER

Results are expressed as Mean value $\pm$ SD (standard deviation). n=6 (NA. of animals in each group), NS – NORMAL SALINE, DZ- DIAZEPAM, NA – AQUEOUS EXTRACT OF NERIUM OLEANDER FLOWERS

GROUPS		ROTAROD	ACTOPHOTOMETER
		Time spent on revolving rod in Rotarod apparatus (secs)	Locomotor activity
GROUP I	CONTROL (NS)	296.67 ± 8.16	401.50 ± 55.67
GROUP II	STANDARD(DZ)	72.00 ± 26.56	159.00 ± 33.65
GROUP III	NA 200mg/kg	202.17 ± 70.56	258.17 ± 19.35
GROUP IV	NA 400 mg/kg	110.33 ± 50.60	212.83 ± 37.04

Table 2: Comparison of results between the groups (control, standard and test groups) using unpaired t test and significance

GROUP COMPARISON	UNPAIRED t TEST & SIGNIFICANCE	
	ROTAROD	ACTOPHOTOMETER
GROUP I VS II	$P < 0.000$	$P < 0.0001$
GROUP I VS III	$P < 0.009$	$P < 0.0001$
GROUP I VS IV	$P < 0.000$	$P < 0.0001$
GROUP II VS III	$P < 0.000$	$P < 0.001$
GROUP II VS IV	$P < 0.48$	$P < 0.002$
GROUP III VS IV	$P < 0.01$	$P < 0.002$



Figure 1: Time spent on revolving rod in Rotarod apparatus (secs)



Figure 2: Locomotor activity in Actophotometer

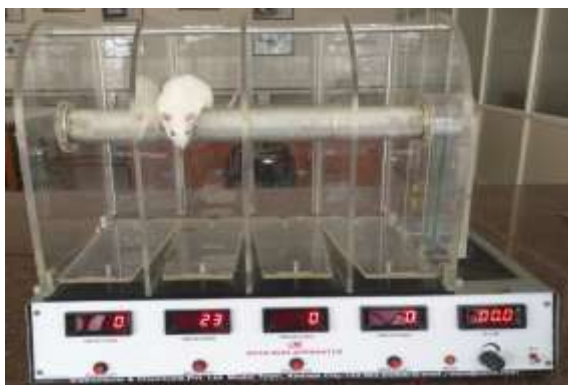


Figure 3: Rotarod Apparatus



Figure 4: Actophotometer

DISCUSSION

Herbal medicines have been extensively used in recent years, owing to their safety profile. The leaves and flowers of *Nerium oleander* has several glycosides. They are known to have cardiotonic, diaphoretic and diuretic, emetic, expectorant properties. Phytochemical tests of NA revealed the presence of alkaloids, tannins, cardiac glycosides, terpenoids, reducing sugars, anthraquinones, flavonoids, saponins⁸. Various studies have shown that the possible mechanism of anxiolytic action of NA could be due to the binding of flavonoids to the GABA A – BZD receptor complex leading to agonistic action.^[9]

The main objective of this study was to evaluate skeletal muscle relaxant activity of aqueous extract of *Nerium oleander* flowers in experimental animals like albino rats. In the present study, we have used two standard models, ROTAROD and ACTOPHOTOMETER to evaluate the skeletal muscle relaxant effect of aqueous extract of *Nerium oleander* flowers.

In Rotarod apparatus, the NO at 200mg/kg and 400mg/kg showed that there was significant reduction in time spent on the rotarod when compared to control

ACTOPHOTOMETER is a model for locomotor activity. Decrease in locomotor activity implies a depressive effect on CNS.^[10] The NA showed dose dependent decrease in locomotor activity compared

to control animals. These observations are similar to the results of the study conducted by M.Rabbani et al.^[11]

Comparison of four groups was done with One Way Analysis of Variance (ANOVA). Multiple comparisons done with Tukey's Post Hoc (table 2). Our study is in correlation with our another study.^[5] Moreover, also with another study by singhal et al.^[12] However, further extensive phytochemical analysis and research is necessary to identify the exact constituents and to know the possible mechanism of action of skeletal muscle relaxant activity of *Nerium oleander*.

CONCLUSION

The present study was designed to evaluate the skeletal muscle relaxant activity of aqueous extract *Nerium oleander* flowers in rats using rotarod and actophotometer models and results are compared with standard drug (Diazepam). Aqueous extract *Nerium oleander* flowers has shown significant skeletal muscle relaxant activity in both rotarod and actophotometer models.

From the above findings, we can conclude that aqueous extract of *Nerium oleander* flowers shown skeletal muscle relaxant activity at both the dose (200 and 400 mg/kg) which is comparable with the standard drugs. However, it requires further studies to evaluate the possible mechanism involved and safety and efficacy of it's use in human beings.

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DECLARATIONS

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Ethical approval: The study was approved by the Institutional Ethics Committee of JJM Medical College (Committee for the Purpose of Control and Supervision of Experiments on Animals, India Ref No. JJMMC/IAEC/01-2016).

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