

EFFECT OF METHANOLIC EXTRACT OF NERIUM OLEANDER ON SKELETAL MUSCLE OF MICE

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ABSTRACT

Objective: The aim of the present investigation was to prepare Methanolic extract leaves of *Nerium Oleander* & evaluate muscle relaxant activity of Methanolic extract using Rota rod test. **Rota Rod**

Method: The test is used to evaluate the activity of drug interfering with motor coordination. In 1956, Dunham and Miya suggested that the skeletal muscle relaxation introduced by a test compound could be evaluated by testing the ability of mice or rats to remain on revolving rod. This forced motor activity has subsequently been use by investigator. The dose which impairs the ability of 50% of the mice to remain on the revolving rod is considered the endpoint. **Results:**

Methanolic extract of leaves of *Nerium Oleander* at dose of 25mg/kg

shows Skeletal Muscle Relaxant Activity was indicated by decrease in fall of time from Rotating rod in mice as compare with control group of animal. But at 50mg/kg dose of extract shows hyper response in mice and fall of time of mice from rotating rod is an increase. As an indications of extract dose not showed Skeletal Muscle Relaxant Activity at higher dose. **Conclusion:** Methanolic Extract of *Nerium Oleander* posse's significant muscle relaxant activity in comparing with control group. The muscle relaxant activity of extract may be due to its CNS-depressant activity due to cardenolides including a new cardenolides, neridginosides and three constituents are nerizoside, neritaloside and odoroside –H.

KEYWORDS: Nerium Oleander, Methanolic Extract, Rota Rod, Muscle Relaxant Activity, Mice etc.

INTRODUCTION^[1,2]

Skeletal muscle relaxants are used to treat muscle spasm & spasticity. Muscle spasm is defined as a sudden involuntary contraction of one or more muscle groups and is usually an acute condition associated with muscle strain (partial tear of a muscle) or sprain (partial or complete rupture of a ligaments).

Drug Showing Muscle Relaxant Activity^[6]

1. Diazepam
2. Haloperidol
3. Benzodiazepines
4. Mepenesin
5. Chlorzoxazone
6. Metocarbamol
7. Quinine

Plant Showing Muscle Relaxant Activity^[8]

1. Nerium Oleander
2. Wedelia Trialbota
3. Ammi Visnaga
4. Chondrodendron Tomentosum
5. Pyrenacantha Staudtii
6. Portulaca Oleracea

MATERIALS AND METHODS

All chemicals and reagents used in the study were of analytical grade Methanol (90%) Loba Chemie Pvt.Ltd, Diazepam Rambaxy Lab.Ltd, Distilled water, Soxhelt Extractor J Sil GlassTM, Rotarod INCO (Instruments & Chemicals (P) Ltd. Ambala.

Animal Used In Research Work

Albino Mice weighing 20- 40 gram were used in the study. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages.

Plant Materials

The leaves of *Nerium Oleander* were collected from local area of Lasurne Tal: Indapur Dist: Pune, Pine Code: 413114 in the month of October 2012. The leaves are dried in shade for few days.

Method of Extraction^[7]

Soxhelt extraction is the process of continuous extraction in which the same solvent can be circulated through the extractor for several times this process involves extraction followed by evaporation of solvent. The vapours of solvent are taken to the condenser and condense liquid is return to the drug for continuous extraction. Soxhelt apparatus is design for such continuous extraction consist of body of the extractor attach with the side tube and siphon tube. The extractor from a lower side can be attached to the distillation flask. Initially for the settling of the powder solvent (Methanol) is allow to siphon once before heating. Fresh activated porcelin pieces are added to the flask to avoid bumping of solvent, vapour passes to the side tube. A siphon is setup as the liquid is reaches. The point of returns and the contents of extraction chamber are transfer into the flask. The cycle of solvent evaporation and siphoning back can be continued as many times as possible without changing solvent, so as to get efficient extraction.

Rotarod Method^[9]

The apparatus consists of 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 3 sections by plastic discs, thereby allowing the simultaneous testing of 3 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. Cages below the sections serve to restrict the movement of the animals when they fall from the roller. Mice with an weight between 25 and 35g undergo a pretest on the apparatus. the test compound are administered intraperitoneally or orally, after interaperitoneal administration are administration the mice are placed on the rotating rod. Compare the fall off time of animals before and after drug treatment.

Experimental Method

For Muscle Relaxant Activity, Albino mice of either weighing between (20-40gms) were divided into four groups. First group (Control) received vehicle only (Distilled Water). Second group received Diazepam in a dose of 4 mg/kg, intraperitoneally. Third and Fourth

groups received ME (25 mg/kg and 50mg/kg, i.p., each). Turn on the Rota-rod. Select an appropriate speed (20-25rpm is ideal). Place the mice one by one on rotating rod. (If, the rod is divided into several compartments, one can place more than one mouse at a time). Note down the “fall of time”, when the mice falls from the rotating rod. A normal mouse generally falls off within 3-5 minutes Inject diazepam in second group animals. After, 30minutes repeat the experiment, note the fall off time. An Alcoholic Extract of *Nerium Oleander* was injected in Third and Fourth groups of animals. Wait for 30minutes. After, 30 minutes repeat the experiment. Note the fall off time.

Table 1: For Controlled Condition (without drug).

Sr. No.	Body Weight (gm)	Volume of drug	Time of Fall in Sec.
1	32	-	300
2	33	-	295
3	32	-	278

Table 2: For Standard Drug (Diazepam- Dose 4 mg/kg).

Sr. No.	Body Weight(gm)	Dose (mg/gm)	Volume of Drug	Time of Fall		% Decrease in time (Sec)
				Before Drug	After Drug	
1	32	0.800	0.32	300	30	99.00
2	33	0.825	0.33	295	29	90.16
3	32	0.800	0.32	278	36	87.05

RESULTS AND DISCUSSION

ME of Leaves of *Nerium Oleander* at dose of 25mg/kg shows Skeletal Muscle Relaxant Activity was indicated by decrease in fall of time from Rotating rod in mice as compare with Control group of animal.

But at 50 mg /kg dose of extract shows hyper response in mice and fall of time of mice from Rotating rod is increase. Indication of extract does not showed Skeletal Muscle Relaxant Activity at higher dose.

Effect of ME Leaves of *Nerium Oleander* on Mice by Using Rota Rod.

Table 3: For Methanolic Crude Extract (Dose-25mg/kg).

Sr. No.	Body Weight(gm)	Dose (mg/gm)	Volume of Drug	Time of Fall		% Decrease in time (Sec)
				Before Drug	After Drug	
1	29.50	0.740	0.29	300	112	62.11
2	33.75	0.843	0.33	295	40	86.44
3	27	0.675	0.27	278	58	79.13

Table 4: For Methanolic Crude Extract (Dose-50mg/kg).

Sr. No.	Body Weight(gm)	Dose (mg/gm)	Volume of Drug	Time of Fall		% Decrease in time (Sec)
				Before Drug	After Drug	
1	23.00	1.15	0.23	300	101	65.17
2	33.01	1.65	0.33	295	96	70.00
3	30.30	1.51	0.30	278	94	62.40

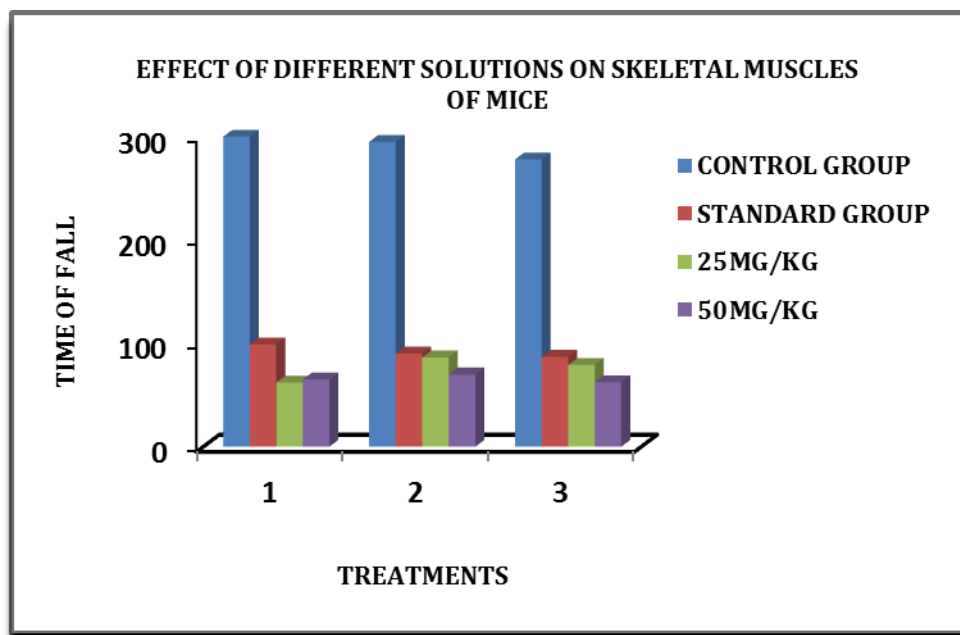


Figure 1: Graph of Time of Fall Vs Treatments.

The muscle relaxant activity was examined for ME of *Nerium Oleander* by using rotarod apparatus in mice the rotarod test on mice caused the increase rotation of rod in control group [receiving only vehicle]. The mice is treated with ME of *Nerium Oleander* has shows skeletal muscle relaxant effect in test group.

It cause induction of skeletal muscle relaxation by directly or indirectly reducing the hyper activity in the nervous centers concerned with the stretch reflexes in spasticity and with the flexion reflex, as in painful muscle spasm or the hyperactive withdrawal reflex.

In the present investigation of Diazepam [4mg/kg i.p.] is used as standard muscle relaxant drug.

Nerium Oleander is well known for its rich glycosides and other chemical contents these are responsible for CNS depressant properties that may contribute to or are mainly responsible for the skeletal muscle relaxant activity.

ME of leaves of *Nerium Oleander* at dose of 25mg/kg shows relaxation of skeletal muscle but at a dose of 50mg/kg of ME of *Nerium Oleander* shows Excitation of animals due to stimulation of Central Nervous System, So higher dose of ME of Leaves of *Nerium Oleander* does not show Skeletal Muscle Relaxant Activity.

CONCLUSION

Based on forgoing observation and result it could be concluded that Methanolic Extract of *Nerium Oleander* posses significant muscle relaxant activity in comparing with control group. The muscle relaxant activity of extract may be due to its CNS-depressant activity due to cardenolides including a new cardenolides, neridginosides and three constituents are nerizoside, neritaloside and odoroside –H.

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