International Journal of Pharmacy Research **Vol. 1, No. 2, 2010**
0976-2167

Acute toxicity and analgesic activity of *Nerium oleander* stem extracts on wistar strain albino rats

Bhatia Tarun *, Makkar Tannu, Gupta A. K., Upmanyu Garima

** PDM School of Pharmacy, Karsindhu, Safidon, Haryana*

Research Article

ABSTRACT

Nerium oleander is used traditionally as an analgesic drug from a long time. This plant is found wildly throughout the world. The extracts of this drug were found safe up to 2000 mg/kg. Therefore 200 and 400mg/kg dose were taken according to OECD guidelines. The ethanolic and aqueous extract shows significant results in tail flick method when compared to Pentazocin which was taken as standard, while in Acetic Acid model ethanolic extract shows more significant ($p < 0.05$) inhibition to writhing induced by acetic acid than aqueous when compared to aspirin. The results suggest the presence of a potent analgesic principle in the extract, which support the folklore use of the plant in relieving pains.

Key words: *Nerium oleander, acute toxicity, Analgesic, Writhing, Tail immersion*

Corresponding Author: Tarun Bhatia, PDM School of Pharmacy, Karsindhu, Safidon, Haryana, India, Tel: +919896602071.
E-.mail: tarun2071@gmail.com

Article Info: Date received: 17 March 2010 **Date accepted : 3 July 2010**

INTRODUCTION

Nerium oleander has many therapeutic uses in different traditional medicine of the world. Different parts of the plant have different therapeutic values. In ethno botanical literature it is mentioned to be effective in the treatment of cardiac illnesses [1] asthma, corns, cancer & epilepsy [2] and also used as diuretic. The leaves and the flowers are cardio tonic, diaphoretic, diuretic, emetic, antibacterial, expectorant and have antiplatelet aggregation activity. A decoction of the leaves has been applied externally in the treatment of scabies, and to reduce swellings. It is beaten into a paste with water and applied to chancres and ulcers on the penis. Oil prepared from the root bark is used in the treatment of leprosy and skin diseases of a scaly nature. The whole plant is said to have anticancer properties. Fresh leaves have been applied to treat tumors .The decoction of leaves and bark has been used to treat syphilis, and used as a gargle to strengthen the teeth and gums and as a nose drop for children. Its various parts are reputed as therapeutic agents in the treatment of swellings, leprosy, eye and skin diseases. The plant has been used in the treatment of cardiac illness, asthma, diabetes mellitus, corns, scabies, cancer and epilepsy. The diuretic and

analgesic effects of polysaccharide present in leaves of Nerium oleander Linn. [3] shown that the same possibilities exist for stem of the plants.

MATERIAL AND METHODS

Plant materia

The Nerium oleander (Apocynaceae) stem was collected from the Karnal, India (GPS coordinates) in June 2008. Botanical identification was confirmed by morphological characteristic from Raw Materials Herbarium and Museum, NISCAIR, DELHI) with accession no. Niscair/RHMD/ Consult/-2008-2009/1080/111 . A voucher specimen no. hcop/pcg / 01763/ 08 of same was submitted to Hindu college of pharmacy, Sonipat, India.

Preparation of Extracts

The stem was dried at room temperature under well-ventilated shade by spreading them uniformly. The dried part was powdered, weighed (300gm) and filled in Soxhlet apparatus for successive solvent extraction with different solvents viz. pet-ether, ethanol and water. Before making extraction with next solvent previous one was dried at a temperature below 600C. Percentage Yield was calculated for each extract after drying it under vacuum [4, 5].

Animals

Wistar strain albino rats of either sex (150-200 g) were procured from Institutional Animal house, Hindu college of pharmacy, Sonipat. Throughout the experimental period, the animals were housed in colony cages. The animals were provided with food (Golden feed) and water ad libitum. They were maintained at a temperature range of 22–25 °C.

Chemicals

Acetyl salicylic acid, Pentazocin was procured from Dabur Research Foundation, Ghaziabad, India. All the chemicals used were of analytical grade.

ACUTE TOXICITY STUDY

Approval of the institutional Animal Ethics Committee was obtained prior to experimentation on animals. Acute toxicity study was conducted according to OECD Guidelines 420. Fixed dose method Test procedure with a starting dose of 2000 mg/kg body weight was adopted. With the starting dose of 2000 mg/kg of each extract was given to 5 animals, and animals were observed for any behavioral change and death for 14 days. [6, 7]

Analgesic activity

Tail immersion test [8, 9]

Young male Wistar strain albino rats (170–210 g body weight) were used. Six groups having six animals in each group were taken. Group one served as control (received 0.9% Normal saline solution) and group two served as standard (received pentazocin at a dose 2 mg/kg) in 0.9% Normal saline solution. Remaining groups received test drug at a dose level of 200 and 400 mg/kg in 0.9% Normal saline olution. Each of the preparation was given in such a manner so that the fluid intake was same in all cases. They were placed into individual restraining cages leaving the tail hanging out freely. Before testing the animals

were allowed to adapt to the cages for 30 min. The lower 5 cm portion of the tail was marked. This part of the tail was immersed in a cup of freshly filled water at exactly 55 °C. Within few seconds, the rat reacts by withdrawing the tail. The reaction time was recorded in 0.5 sec. units using a stopwatch. After each determination, the tail was carefully dried. The reaction time was determined before and periodically after oral administration of the test substance (after 0, 15, 30, 60, 120 and 180 min.). The cut off time of the immersion was 15 sec. The withdrawal time of untreated animals was between 1 and 5.5 sec. A withdrawal time of more than 6 sec. therefore was therefore regarded as a positive response.

Acetic acid induced writhing method [10]

Wistar strain albino rats of either sex were used. Six groups having six animals in each group were taken. Group one served as control (received 0.9% Normal saline solution) and group two served as standard (received aspirin at a dose 100 mg/kg) in 0.9% Normal saline solution. Remaining groups received test drug at a dose level of 200 and 400 mg/kg in 0.9% Normal saline solution. Each of the preparation was given in such a manner so that the fluid intake was same in all cases. Rats were made to writhe by an i.p. injection of 0.6% v/v aqueous acetic acid (0.1 ml/kg). Test substance was administered 30 min. before injection of acetic acid. Animals were kept under observation immediately after injection of acetic acid for 30 min. The number of writhes was recorded as number of abdominal contractions, trunk twist response and extension of hind limbs. Percentage inhibition of writhing was calculated.

STATISTICAL ANALYSIS

Results of all the above methods are expressed as Mean SEM. Total variation in asset of data was estimated through one way analysis of variance (ANOVA) followed by Dunnett's test. Values of $P<0.01$ were considered statistically significant.

RESULTS

Acute Toxicity Study

It can be revealed from the table no. 1 that both the ethanolic and aqueous extracts of N. oleander were found to be safe up to 2000 mg/kg body weight. Therefore the dose of 200 mg (1/10th) and 400 mg (1/5th) was selected for all in vivo studies.

Tail Immersion method

From the table no.2 data it is revealed that the ethanolic and aqueous extract at the dose level of 400mg/kg provided significant response ($P<0.05$) at 120 min. Analgesic study by tail-immersion test provided the evidence for central mechanism which is also exhibited by the standard drug for relieving the pain.

Acetic acid induced Writhing method

From the table 3, it is concluded that ethanolic extract shows significant analgesic activity at 200mg/kg and 400mg/kg (at $p<0.5$) as compared to aqueous extract.

DISCUSSION

This study is aimed at evaluating the scientific basis for the traditional use of N. oleander against rheumatic pains. The results showed that the ethanolic and aqueous extract of plant have significant analgesic effect

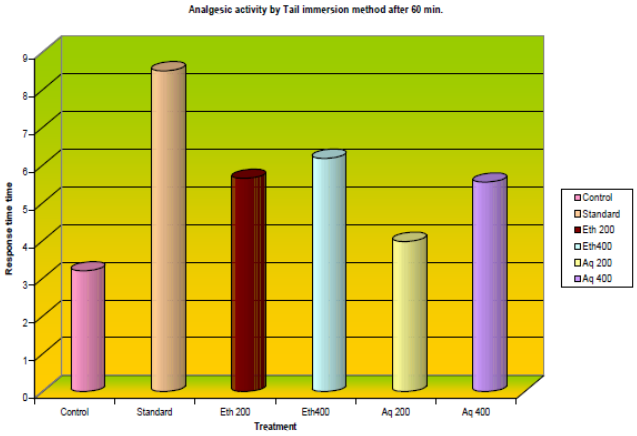
(p>0.05) and in acetic acid induced writhing ethanolic extract has shown significant inhibition. From the current study it is revealed that the writhing induced by chemical substances is due to sensitization of nociceptors by Prostaglandins. The ethanolic extract at the dose level of 400mg/kg showed significant inhibitory activity on the writhing induced by acetic acid model when compared to control.

Acknowledgement

The author is thankful to Kuldeep Singh for his technical assistance and help in writing manuscript and institutional head for providing all facility to carryout work.

REFERENCES

1. Fumiko Abe, Tatsuo Yamauchi. Cardenolide trioside of oleander leaves, Phytochemistry, 1991, 31(7), 2459-2463.
2. Encyclopedia of medicinal plants, 1984.
3. Ming Zao, Limming Bai, Liyan wang, Asami Toki. Three new triterpenes from Nerium oleander and biological activity of isolated compounds, Journal of natural Product, 2005, 68,198-206.
4. Harborne, J. B. Phytochemical method: A guide to modern techniques of plant analysis. Chapman and Hall, New York, 2005, 2nd edition, 6, 11, 63.
5. Mukherjee, P.K., Quality control of herbal drugs 2003, 2nd Edition, Published by business horizons, New Delhi, 9, 15, 536, 559, 564.
6. Basu S.K., Arivukkarasu. Acute Toxicity and diuretic activity of Rungia rapens aerial parts in rats, Fitoterapia 2006, 77, 83-85.
7. OECD guidelines for testing of chemicals, Acute toxicity studies-fixed dose procedure, Proposal for a new guideline 425, version, 2004, 1, 14.
8. Lingadurai Sutharson. Anti-inflammatory Anti-nociceptor activity of methanolic extract of leaves of Florbunda wallich, African Journal Traditional CAM 2007, 4(4), 411-416.
9. Vogel H. G. Drug Discovery and Evaluation 2002, 2nd edition, 873, 696, 697.
10. Patro P., Sahu P.K. Anti inflammatory and analgesic activity of aqueous extract of stem bark of Somida febrifuga, Indian Journal of Natural Products 2008, 24(4), 24-27.



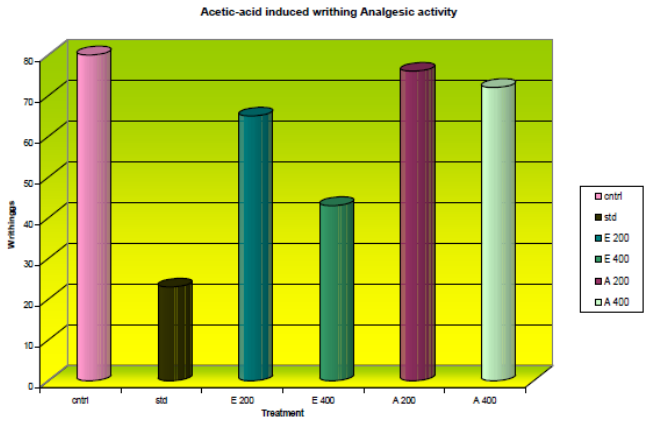


Table 1: Acute toxicity study of extracts of *N. oleander*

Treatment (Extracts used)	No. of animals used	No. of animals recovered*		
		After 24 hr.	After 72 hr	After-14 days
Control	5	++ + + +	+ + ++ +	+ + ++ +
Ethanolic	5	+ + + + +	+ + + + +	+ + ++ +
Aqueous	5	+ + + + +	+ + + + +	+ + ++ +

* +ve =Recovered animals -ve =Dead animals

Table No2: Analgesic activity by Tail Immersion method

Treatment	Dose mg/kg	Time taken to flick the tail (s)					
		0 min.	15min.	30 min.	60 min.	120 min.	180 min.
Control	-	3.16±0.18	3.16±0.24	3.16±0.24	3.20±0.18	3.16±0.18	3.16±0.18
Pentazocin	2	3.33.±0.14	6.72±0.15**	7.83±0.19**	8.50±0.19**	8.72±.099**	8.83±0.249**
Ethanol	200	3.16±0.71	4.66±0.04	5.23±0.15	5.65±0.068	5.23±0.04	5.50±0.06
	400	3.16±0.71	4.56±0.04*	5.25±0.14*	6.18±0.46*	6.50±0.06*	6.45±0.45*
Aqueous	200	3.16±0.12	3.33±0.09	3.62±0.04	3.98±0.04	3.7±0.09	3.90±0.07
	400	3.33±0.08	4.67±0.59*	4.96±0.04*	5.55±0.07*	6.05±0.11*	6.16±0.04*

Values are expressed in mean ± S.E.M., n=6, * Significant at $p < 0.05$, ** Significant at $p < 0.01$ Vs control, Dunnet's test,

Table No 3: Analgesic activity by Acetic acid induced writhing response

Treatment	Dose (mg/kg)	Number of writhing	% Inhibition of writhing
Control	-	80±2.082	-
Aspirin	100	23±2.422**	71.25**
Ethanol	200	65±2.033**	18.75**
	400	43±1.211**	46.25**
Aqueous	200	76±1.915	5
	400	72±3.173	10

Values are expressed in mean ± S.E.M., n=6, * Significant at $p < 0.05$,
** Significant at $p < 0.01$ Vs control, Dunnet's test.