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TOXIC EFFECT AND ORAL ACUTE LD_{50} STUDY OF *NERIUM OLEANDER* IN MALE GUINEAPIGS

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ABSTRACT

The toxic effect of *Nerium oleander* was studied in 36 male adult guineapigs during the period from July to December 1994. These 36 animals were divided into six equal groups (A to F), each consisting of six animals. Each animal of groups B to F was administered with a single oral dose of crude watery extract of sheath oleander @ 300, 450, 600, 750 and 900 mg / kg body weight, respectively whereas animals of group A served as control. Each of the experimental animals was carefully observed and the toxic signs recorded as nausea, anorexia, dullness, depression, restlessness, abdominal pain, salivation, reluctant to move, tremor, resting of chin on the ground, respiratory distress, paralysis of the limbs, recumbency, convulsion followed by death with characteristic groaning. It may be concluded that the lowest dose 300 mg / kg body weight is non lethal to the male guineapigs and the dose of 450, 600, 750 and 900 mg / kg body weight caused 17%, 50%, 83% and 100% mortality, respectively and the LD_{50} is 540 mg / kg body weight.

Key words: *Nerium oleander*, toxic signs, oral acute LD_{50}, guineapigs

INTRODUCTION

Nerium oleander belongs to the *Apocynaceae* family together with the digitalis species in a plant originating from the mediterranean basis and some parts of Asia. Its poisoning is well known in man and has been also reported in domestic and wild animals (Mahin *et al.*, 1984). Poisoning occurred due to overdose or misuse of plants or plant products. The plant oleander is extremely toxic in parts, green or dry, to all classes of livestock and man. Leaves have been shown deadly as little as 0.005% of the animal's weight in horses and cattle and 0.015% in case of sheep. A single leaf is considered potentially lethal to the man. Loss of human life, sometimes involving large number of poisons during military operations, has repeatedly occurred when meat was rosted while skewered oleander branches. Research data on *Nerium* spp. in Bangladesh are scanty, although *Nerium* spp. are the ornamental plants available elsewhere in the country and causes of poisoning are accidental but common. The aim of the study is to investigate the toxic effect, time and determine acute oral LD_{50} of *Nerium oleander* in guineapig.

MATERIALS AND METHODS

Forty apparently healthy adult male guineapigs (*Cavia cobaya*) of nondescript breed and weighing between 700-900 g obtained from the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, were used in this study. All the guineapigs were kept under observation in order to acclimatize to the new environmental condition for one week prior to the commencement of the experiment. All the guineapigs were maintained under good housing conditions and were provided with normal feed and tap water *ad libitum*.

Thirty grams fresh mature leaves of sheath oleander were collected from plant and finely grinded in a mortar and made the volume up to 300 ml. The mixture then filtered through a muslin cloth and the filtrate used as crude watery extract of sheath oleander for administration to the guineapig. A preliminary trial was conducted in four male guineapigs to assess the approximate lethal, sub-lethal, and toxic doses of crude watery extract of sheath oleander. Thirty-six adult male guineapigs were randomly divided into six groups and each group comprising of 6 animals. The groups were designed as A, B, C, D, E and F. All the animals were weighed carefully and kept group-wise in 6 cases. Each animal of groups B to F was administered crude watery extract of sheath oleander in single oral doses of 300, 450, 600, 750, and 900 mg / kg body weight respectively, whereas animals of group A served as control. Guineapigs were carefully observed for the appearance of any toxic signs, lethality, nature, time of appearance of various toxic manifestations, and the mortality rate recorded in each group of animals. Oral acute LD_{50} was determined according to the experimental design adopted by Singh (1981).

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STATISTICAL ANALYSIS

All the data were analyzed statistically between control and treated values by using Student's '*t*' test (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

The single oral administration of crude watery extract of sheath oleander in doses of 300, 450, 600, 750, and 900 mg / kg body weight induced a varying degree of toxicity and mortality. The toxic signs exhibited by crude watery extract of sheath oleander were nausea, anorexia, dullness, depression, restlessness, abdominal pain, salivation, reluctant to move, tremor, resting of chin on the ground, respiratory distress, paralysis of the limbs, recumbency, convulsion followed by death with characteristic groaning. The detailed of the nature and time of onset of various toxic signs and of mortality in these animals are summarized in Table 1. Almost similar types of toxic signs have also been reported by other workers in Karabi poisoning in animals (Sobhan, 1980; Pinto *et al.*, 1981; Anderson *et al.*, 1983 and Alfonso *et al.*, 1994).

Table 1. Nature and time of onset of toxic signs in male guineapigs administered with a single oral dose of crude watery oleander extract

S / N	Signs	Groups					
		A (n = 6) (0 mg)	B (n = 6) (300 mg)	C (n = 6) (450 mg)	D (n = 6) (600 mg)	E (n = 6) (750 mg)	F (n = 6) (900 mg)
1.	Nausea	–	2-3 h	1-2 h	20-30 min	10-12 min	5-7 min
2.	Anorexia	–	2-4 h	2-3 h	30-40 min	10-12 min	5-8 min
3.	Dullness	–	3-4 h	2-4 h	35-40 min	11-12 min	6-8 min
4.	Depression	–	4-6 h	3-4 h	40-45 min	12-15 min	7-8 min
5.	Restlessness	–	4-6 h	3-5 h	1-1.1/2 h	12-15 min	7-9 min
6.	Abdominal pain	–	4-7 h	3-5 h	1-2 h	13-16 min	8-10 min
7.	Salivation	–	4-7 h	4-6 h	2-3 h	13-16 min	9-10 min
8.	Reluctant to move	–	5-8 h	5 -6 h	2-3 h	14-17 min	10-12 min
9.	Tremor	–	–	8-10 h	4-6 h	15-20 min	12-14 min
10.	Resting chin on the ground	–	–	12-16 h	6-8 h	16-20 min	13-15 min
11.	Respiratory distress	–	–	–	10-12 h	17-21 min	14-18 min
12.	Paralysis of both fore and hind limbs	–	–	11-14 h[b]	20-24 min[c]	20-24 min	15-20 min
13.	Recumbency	–	–	20 h[a]	12-15 h[b]	22-50 min[c]	15-25 min
14.	Convulsion	–	–	–	12-16 h[b]	25-60 min[c]	15-30 min
15.	Groaning	–	–	–	13-18 h[b]	30-90 min[c]	20-52 min
16.	Death	–	–	30 h[a]	13-23 h[b]	35-150 min[c]	52 min

n = No. of guineapigs used, ( ) = *Nerium oleander* @ / kg body weight orally, a = one guineapig, b = three guineapigs, c = five guineapigs.

At 300 mg / kg body weight dose of crude watery extract of sheath oleander, all the animals displayed mild to moderate toxic signs without causing any mortality. This dose (300 mg / kg) of crude watery extract of sheath oleander was found non-lethal toxic for male guineapigs. Crude watery extract of oleander @ 450 mg / kg body weight was lethal to one of six guineapigs. In this group (group C), five animals showed moderate toxic signs and one animal showed severe toxic signs leading to death at 30 h of receiving the crude watery extract causing 17% mortality. Rest five animals recovered within 48-72 h after receiving the extract.

In group D, all the animals receiving 600 mg of the green leaves / kg body weight showed moderate to severe toxic signs. Three of six animals died showing convulsion and characteristic groaning within 13-23 h following administration of the extract causing 50% mortality. Rest of the animals recovered gradually within 72 h of medication.

The animals of group E receiving 750 mg green leaves / kg body weight exhibited severe toxic signs, which appeared within 10-12 minutes of administration of extract. Five animals died within 35-150 minutes following medication. The mortality being 83% and one animal of this group survived and recovered to normal within 72 h of medication. The animals of group F receiving 900 mg of the leaves / kg body weight showed per acute toxic signs followed by death within 52 minutes of administration of the crude watery extract showing 100% mortality.

The toxic signs showed by all guineapigs of group B to D were almost similar with increasing the dose. Shahidullah *et al.* (1990) reported that *Nerium indicum* @ 800 mg / kg body weight was lethal to guineapig and the animal died within 6 h of Rakta Karabi extract feeding. In the present study, although lethal dose of *Nerium oleander* was found to be slightly higher (900 mg / kg body weight) but here all the guineapigs died within 52 minutes of feeding the extract indicating that the *Nerium oleander* cause rapid death than *Nerium indicum.*

Crude watery extract of sheath oleander in single oral doses of 300, 450, 600, 750, and 900 mg / kg body weight was used for determination of oral acute LD_{50} in adult guineapigs. Based on the mortality rate recorded following above mentioned doses, oral acute LD_{50} was found to be 540 mg / kg body weight for adult male guineapigs (Table 2).

Table 2. Oral acute LD_{50} of crude watery extract of the green leaves of sheath oleander in adult male guineapigs

Groups of animals	No. of animals	Dose (mg/kg bwt)	Dose difference	No. of death	Mean*	Product**
A	6	0	0	0	0	0
B	6	300	0	0	0	0
C	6	450	150	1	0.5	75
D	6	600	150	3	2.0	300
E	6	750	150	5	4.0	600
F	6	900	150	6	5.5	825

*The interval mean of the number of dead animals, **The product of the interval mean and the dose difference.

$$LD_{50} = \text{Highest dose} - \frac{\text{Sum of the product}}{\text{Total No. of groups}}$$

$$= 900 - \frac{1800}{5}$$

$$= 900 - 360 = 540 \text{ mg / kg Bwt}$$

Shahidullah *et al.* (1990) reported that the oral acute LD_{50} of *Nerium indicum* leaves was 640 mg / kg body weight in guineapigs. The variation in LD_{50} depends on sex, species and route of administration of extract. The LD_{50} values of Karabi extract vary considerably in different species of animals and birds. The variation of LD_{50} values between Shahidullah *et al.* (1990) report and the present study may be due to species difference of the plant i.e., *Nerium indicum, Nerium oleander* and other certain factors of unknown nature.

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