Indian Journal of Experimental Biology
Vol. 42, October 2004, pp. 1003-1006

Toxicity of *Nerium indicum* Miller seed extract on bandicoot rat, *Bandicota bengalensis* Gray

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Received 13 November 2003; revised 11 June 2004

Crude extract (12.5 ml/kg) of *N. indicum* seed gave 100% mortality of *B. bengalensis*. Humanness assessment study revealed that this plant orgin chemical caused low pain and sufferings to the target pests.

Keywords: Biopestiside; *Nerium indicum*; Seed extract; *Bandicota bengalensis*

Rodents form an important component among the vertebrate pests, affecting the agricultural production in many developing tropical countries[1,2]. Rodents are highly adaptable, prolific breeders and can cope up with new environments, new foods and adjust to new associateship with a striking swiftness, and hence, it is difficult to check down their population for longer periods either by any cultural methods or natural enemies or synthetic chemical agents[3]. In recent years there has been concern about the use of bio-pesticides in controlling pests. Plant substances were traditionally used as rodenticide or repellent but have now been almost completely replaced by chemicals. Seeds of Mexican poppy (*Argemone mexicana*) were used to kill roof rat (*Rattus rattus*)[4]. In Maharastra, the tribals used oleander (*Nerium indicum*) plant parts as rat poison[5]. During Peninsular Wars, some of Wellington's men were fatally poisoned after eating meat cooked on oleander skewers[6]. Oleander (*Nerium indicum* Miller) is a popular and commonly grown tropical shrub. This shrub is too small for the timber to be of any commercial value. Although its toxicity has been long known, accidental poisoning from the use of the wood continues to occur[7]. All parts of the plants are reported to contain poisonous substances[5,8,9]. Seeds contain the toxic substances Neriodorin (soluble in chloroform) and Neriodorein (soluble in water) which are powerful heart poisons[9]. Leaves and stem contain dombonitol, root bark contain 2,4-dihydroxy acetophenone and 4-hydroxy 2-acetophenone, which cause loss of muscular control, breathing troubles and paralysis[10]. Keeping this information in view, the present study has been undertaken to know the basic toxicity of seed extract of Indian oleander (*Nerium indicum*) on the bandicoot rat, *Bandicota bengalensis* Gray in laboratory.

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Materials and Methods

Bandicoot rats collected from local agricultural fields by burrow digging method, were brought to the laboratory and lodged individually in cages. They were fed with laboratory feed (whole-wheat flour 65%, perched Bengal flour 15%, rice flour 10%, sugar 5%, ground nut oil 2.5% and water 2.5%) for 15 days in order to acclimatise to the laboratory conditions.

Collection of oleander seeds and extraction of poison—Matured seeds of oleander were collected locally. To obtain crude extract the kernels were removed from the seeds and allowed to shade-dry for five days. Thereafter the kernels were finely grounded. The fine powder was mixed with distilled water in a ratio of 1:7.5. This mixture was thoroughly stirred with a glass rod with an interval of 10 min for 1 hr to dissolve steroidal compounds in the water. The mixture was then centrifuged at 2000 rpm for 10 min and the supernatant was taken for experiment.

Toxicity trial—Seed extract was orally administered by stomach gavage needle at different dose levels (5, 7.5, 10 and 12.5 ml/kg body weight). Twelve animals (irrespective of sexes) were tested for each dose level. Before commencing the experiment, the test animals were starved for 24 hrs. Food and water were provided after one hour of dosing. Percent mortality and hours to death at each dose level were noted.

Humanness assessment—Pain and suffering of the experimental animal due to poisoing was assessed by the method of Johnson and Prescott[11]. In this method the degree of pain and suffering was measured indirectly by recording the behavioural (lying, moving, feeding, drinking, gait and crouching) and morphological changes. After oral dosing, all animals were observed every 30 min for two duration (0600 – 0800 hrs and 1800 – 2000 hrs) each day and then every 30 min for 24 hrs during the predicted sickness period (such animals considered as moribund animal) in order to monitor behavioural changes more closely. At each 30 min observation, activities include feeding drinking, gait (changes in walking) and crouching (standing with bent legs and weight over forelegs, back hunched, abdomen tucked up and often with head down) and clinical signs of poisoning were noted. Then these data were transformed into percentage.

Statistical analysis—Analysis of Variance (One way ANOVA) and Bonferroni post-hoc tests were used to determine timing of feed intake changes.

Results and Discussion

Per cent mortality of *B. bengalensis* at different intervals after oral intubation of seed extract is given in Table 1. Hundred per cent mortality of *B. bengalensis* occurred with the highest test dosage of 12.5 ml/kg of crude extract of *N. indicum*. At lower dosages of 10, 7.5 and 5 ml/kg the *B. bengalensis* registered 50, 25 and 10% mortality, respectively. Mortality started after 24 hr of poisoning with 10 and 12.5 ml/kg dose. Observed signs of poisoning were sedation, paralysis, sluggishness, feeble or no muscular movement and abdominal contractions. Similar signs have also been reported by Rastogi *et al.*[12] in human beings after accentental intake of oleander seeds. According to Sambandhamurthy and Subrahmanyam[5] sterol and other steroidal compounds present in the *Nerium* spp are responsible for loss of muscular control, breathing trouble and paralysis.

Table 1—Mortality of *B. bengalensis* at different time intervals after oral feeding of seed extraction of *N. indicum*

Dose (ml/kg)	Mortality (%) after			
	24 hr	48 hr	72 hr	96 hr
5.0	0	0	0	10
7.5	0	0	25	25
10.0	25	50	50	75
12.5	50	75	100	100

N. indicum caused a significant decrease in the levels of protein, free amino acid, DNA and RNA in the nervous tissue of *Lymnaea acuminata*[13]. Rastogi *et al.*[12], following the post mortem examination reported that the poison had affected the vital organs such as brain, heart, lungs, stomach liver, spleen, intestine and kidney. Further, they observed that about 1½ hour after the poison had been taken the patient was apparently senseless and unable to answer questions, the pulse was naturally slow and soft but regular.

Humanness assessment—Humanness is an ambiguous term that is difficult to define, however it inferrs to minimization of pain, distress and discomfort during the killing of animals through control programmes[11]. Behavioural changes of rat due to poisoning were observed to know the humanness of oleander's seed poison.

Feed intake of bandicoot rats significantly declined after poison ingestion in all doses. Intake of feed started declining significantly ($P<0.05$; Bonferroni post-hoc test) one day after dosing with all the test concentrations indicating quick action of poison which lasted up to maximum of four days. During these days the bandicoots became dull and less reactive to stimuli and showed increased lying with decreased moving. Behaviour of untreated rats (control) did not change throughout the experimental period (Fig. 1). During initial stages the treated animals showed inactiveness even at the lower dose levels (Fig. 1). Throughout prolonged inactivity, the treated rats showed abnormal postures compared to control rats. After 24 hr of poisoning, there was a corresponding drop in amount of time spent in moving at all dose levels (Fig. 1) and the rats showed increased lying. However after 72 hr of treatment with higher doses (10 and 12.5 ml/kg) the test animals showed slight decrease in lying and corresponding increase in crouching. Clinical signs *viz.*, sedation, paralysis, sluggishness, feeble or no muscular movement etc were observed after 48 hr of poisoning in some rats. Animals with these clinical symptoms were considered as moribund and were continuously observed till death. They were also observed crouching prostrate for 2 hr before death. Littin *et al*[14] and Saravanan *et al.*[15] have reported similar observation during sickness in rodents treated with anticoagulant rodenticides. According to these authors increase in timing of crouching and gait for longer periods indicate the pain of the target animal. However in the present study the animals, spent less

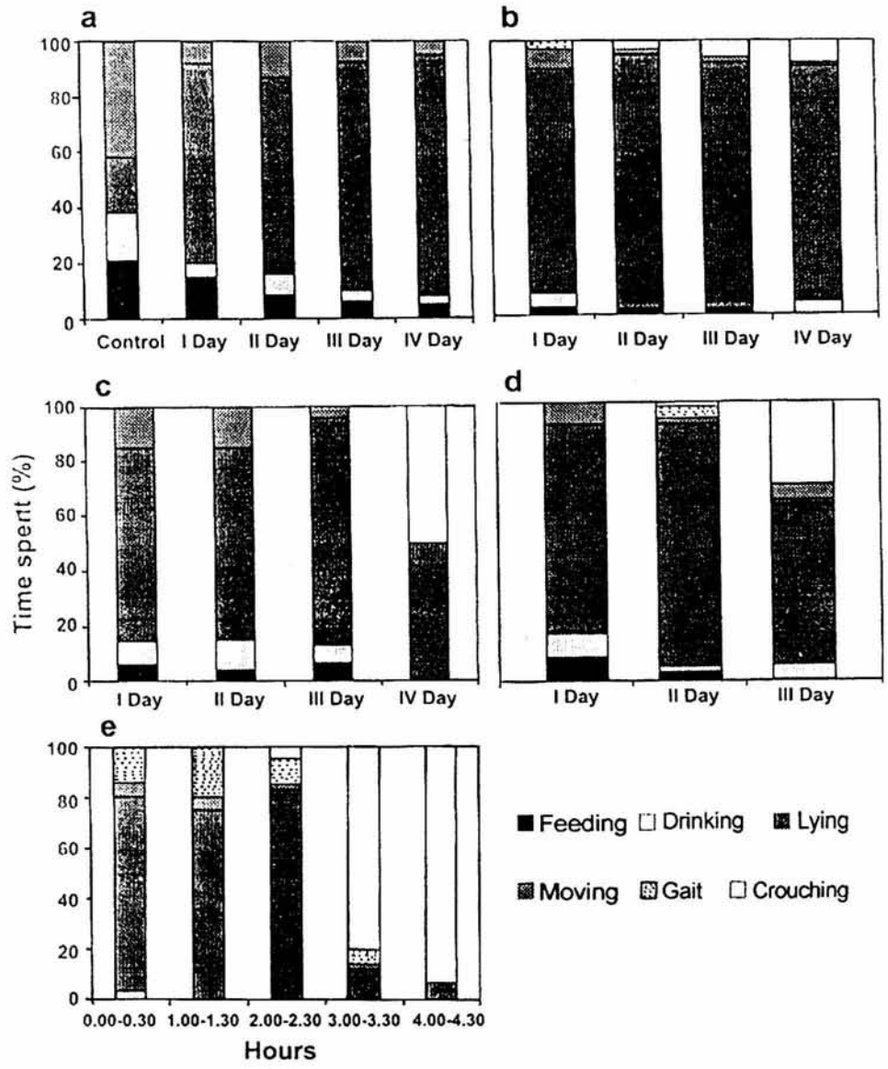

Fig.1—Percentage of time spent performing behaviours each day by the rats after poison ingestion (a: 5 ml/kg; b: 7.5 ml/kg; c: 10 ml/kg; d: 12.5 ml/kg; e: moribund)

time for crouching and gait and spent more time on lying which indicates that the test poison does not give more pain while killing whereas death due to chemical rodenticides yield more pain and more external symptoms such as bleeding and paralysis[14]. Therefore it can be suggested that the seed extract of *N. indicum* may be used as an effective bio-rodenticide with suitable bait bases.

Acknowledgement

Thanks are due to Ms. Jayanthi for valuable suggestions and Dr S Thiyagarajan for computer facilities.

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