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Laboratory evaluation of scilliroside used as a rodenticide against the lesser bandicoot rat, *Bandicota bengalensis*

BY JOE E. BROOKS AND PE THAN HTUN
Rodent Control Demonstration Unit, World Health Organization, PO Box* 14, *Rangoon, Burma*

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SUMMARY

The toxicity and efficacy of the acute rodenticide scilliroside was evaluated in the laboratory against the lesser bandicoot rat, *Bandicota bengalensis*. The acute oral LD50 and LD95 doses for males were 0·8 mg/kg and 2·5 mg/kg respectively, and for females were 0·5 mg/kg and 1·6 mg/kg, respectively. When caged bandicoots were given a choice between plain and poison baits, the optimum concentration of scilliroside was found to be 0·05%. Symptoms of poisoning appear from 22 to 34 min after feeding starts and the latency pattern indicated an abrupt ceasing to feed at these points. Death occurred from 2 h to as long as 6 days after poisoning, following prolonged convulsive seizures. There appears to be aversion to scilliroside at all concentrations in food baits. Maximum mortality attained on free-choice feeding on scilliroside was 90%. Despite these disadvantages, the material may have merit as an alternative rodenticide to zinc phosphide where acute toxicants are to be used.

INTRODUCTION

Red squill, a product from the squill plant, *Urginea maritima* (Baker) L., is one of the oldest and most widely known of the acute rodenticides. The rodenticidal properties were known since antiquity, both the Egyptians and Grecians describing its uses (Stoll, 1954). It is considered one of the safest poisons since it is not readily eaten by animals other than rodents and the crude preparation has natural emetic actions and is regurgitated by man and domestic animals (Pollitzer, 1954).

Scilliroside is the purified, stabilized active rodenticidal component of the red squill plant. The material was first isolated and described by Stoll & Renz (1941, 1942) and its tentative structure defined by Stoll, Renz & Helfenstein (1943). The basic toxicological and pharmacological properties were described by Rothlin & Schalch (1952) and Dybing, Dybing & Stormorken (1952). Scilliroside is a cardio-

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toxic glucoside, chemically known as 6B-(acetyloxy)-3B(B-D-glucopyranosyloxy)-8, 14-dihydroxy-bufa-4,20,22-trienolide. Previous published reports on the evaluation of scilliroside as a rodenticide are few (Barnett *et al.* 1949; Maddock & Schoof, 1970; Ogushi & Tokumitsu, 1972; Tanaka, 1973), thus there is little information on which to judge its value as a rodenticide for use in Southeast Asia.

We examined the rodenticidal properties of scilliroside as part of a series of laboratory evaluations of several acute rodenticides against the lesser bandicoot rat, *Bandicota bengalensis* (Brooks & Htun, 1978; Htun & Brooks, 1979).

MATERIALS AND METHODS

The rats

Bandicoot rats were captured in wooden, locally made live-traps, usually baited with dried fish. Animals were returned alive to the laboratory while still in the traps. Rats used in cage tests were run into dark cloth bags, weighed, sexed and dusted with an insecticide of low mammalian toxicity to kill ectoparasites. The insecticide showed no apparent adverse effects upon the rats. Animals were caged individually and given a locally milled laboratory meal diet with free access to water and allowed to acclimatize to cage conditions for 3 weeks. Animals used for intubation studies were run out of the traps into dark cloth bags, sexed, weighed and, without anaesthesia, given a dose of scilliroside (using the method described by Redfern, 1971). They were then caged, given food and water and observed for poisoning symptoms and mortality for up to 6 days.

Acute oral toxicity determinations

Scilliroside, supplied as the 20% concentrate by the manufacturer, was found to be easily soluble in propylene glycol at concentrations up to 1%. Stock solutions of 0·1–0·5% were diluted in order to obtain dosages ranging from 0·312 mg/kg, to 10 mg/kg, each dose double the lower one. One ml of dissolved scilliroside was given for each 100 g of body weight. The dissolved scilliroside was administered by means of a syringe and an 18-gauge ball-tipped needle, 80 mm in length. Control animals received propylene glycol only in equivalent amounts. The results of the acute toxicity assay were analysed by the method of Litchfield & Wilcoxon (1949).

Efficacy determination

Caged bandicoots were given a free choice between two food cups containing identical food for several nights. If food consumption was normal, then scilliroside was added to the food in one of the cups and offered for two additional nights. Food cups were alternated in placement each night and were staggered alternately from cage to cage when first placed to avoid the bias of position feeding preference. Scilliroside was offered in foods in concentrations ranging from 0·015% to 0·25% by weight. Both dry meal baits and moist baits consisting of boiled rice/ripe banana were used. All amounts consumed were measured to the nearest 0·1 g and spilled foods were caught on papers underneath each cage and accounted for in calculating daily consumption. Poisoning symptoms and mortality were observed for up to 6 days from the start of the poisoning trial.

Latency

Observations on latency (the period between the beginning of feeding and its termination due to the apparent onset of poisoning symptoms) were carried out by offering individually caged rats a no-choice feeding upon laboratory meal containing scilliroside at a 0·05% concentration. In the evening, animals were observed quietly from nearby and the times and duration of feedings were noted. The animals had been previously trained to feed in the evening under a red lamp.

RESULTS

Acute oral toxicity

The acute oral LD50 and LD95 doses of scilliroside are given in Table 1. The lesser bandicoot rat is extremely susceptible to scilliroside, males showing greater resistance than females. At high doses (10 mg/kg and 100 mg/kg) death was observed as early as 60 min after dosing. Some animals became quiet and lethargic within 30–60 min. The mean time to death varied from 13·2 h at 100 mg/kg to 53 h at a dose of 0·3 mg/kg.

Spasmodic convulsions began as early as 1–2 h in animals receiving doses of 100 mg/kg. At lower doses, convulsions were seen the following morning, sometimes continuing for up to 72 h before death, while other animals showed a decreasing frequency of convulsive seizures and eventually recovered. At autopsy, the prominent and consistent finding was pulmonary haemorrhage (77% of all animals). The same was observed with bait-fed animals.

Latency

The time between the start of feeding and the onset of poisoning symptoms that stop feeding are summarized in Table 2. In direct observations of lesser bandicoots at nightly feedings the minimum time for the duration of the feeding period was 22 min and the maximum time was 34 min. Two animals were noticed to stop feeding abruptly at 22 and 30 min with a sharp upward jerk of the head. After the initial feeding period no animal returned to the food cups, contrary to previous observations of lesser bandicoots feeding on pyrinuron and zinc phosphide (Brooks & Htun, 1978; Htun & Brooks, 1979). After feeding stopped, animals groomed themselves and then sat or lay quietly for a period of 2–3 h. They then began exhibiting the hunched forward posture described by Rothlin & Schalch (1952). Convulsive seizures began as early as 2·5–3 h after feeding stopped.

Efficacy determinations

The observed mortality of both sexes when offered scilliroside free-choice in foods at several concentrations is summarized in Table 3. Varying the concentration from 0·015% to 0·25%, an almost 17-fold difference, had virtually no effect upon mortality. The combined-sex mortality at 0·015% concentration was 78·9% and at 0·25% concentration was 80%. The optimum concentration of scilliroside was judged to be 0·05% in both dry meal and moist foods, since on two trials out of three at this concentration, a 90% mortality resulted.

Table 1. *Acute oral toxicity of scilliroside to the lesser bandicoot rat,* B. bengalensis

	Males	Females
N	41	51
Mean weight (±s.e.), g	325·1 ± 15·5	307·6 ± 11·5
Range	170–471	158–474
LD50	0·80 mg/kg	0·52 mg/kg
(95% C.L.)	(0·5–1·26)	(0·36–0·76)
LD95	2·5 mg/kg	1·56 mg/kg
(95% C.L.)	(1·1–5·6)	(0·8–3·2)

Table 2. *Observations on duration of feeding by* B. bengalensis *on bait containing* 0·05% *scilliroside*

Sex	Weight (g)	Time spent in feeding (min)	Duration of feeding period (min)	Amt of food consumed (g)	LD50s consumed	Result (18 h)
F	383	20	22	3·1	7·7	Dead
F	493	22	22	2·7	5·2	Dead
F	371	25	30	2·7	6·0	Dead
M	588	34	34	7·3	7·8	Dead
M	466	28	28	2·6	3·5	Dead
Means	460	25·8	27·2	3·7	6·2	

Table 3. *Observed mortality and mean intake of scilliroside by lesser bandicoot rats when offered foods free-choice for 2 days*

Type of Food	Conc. of Poison (%)	No. of animals M	No. of animals F	Mean weight (g)	Mean dosage of active ingredient consumed (mg/kg)	Mortality M	Mortality F
Lab meal	0·015	5	5	334·1	1·3 ± 0·2	5/5	4/5
Lab meal	0·015	5	4	309·4	1·0 ± 0·3	3/5	3/4
Lab meal	0·020	2	16	329·1	0·7 ± 0·1	2/2	12/16
Lab meal	0·025	5	6	340·7	1·3 ± 0·3	3/5	4/6
Lab meal	0·050	5	5	346·0	0·9 ± 0·3	4/5	5/5
Lab meal	0·050	5	5	313·5	1·0 ± 0·4	2/5	3/5
Lab meal	0·250	5	5	320·6	5·2 ± 1·4	3/5	5/5
Rice/banana	0·025	5	5	383·5	3·3 ± 1·4	4/5	4/5
Rice/banana	0·050	5	5	317·0	3·1 ± 0·8	4/5	5/5
Rice/banana	0·100	2	8	289·2	2·3 ± 0·8	0/2	7/8
		44	64			30/44	52/64
						68·2%	81·3%

An analysis of the survivors (14 males and 12 females) indicated that 10 males had consumed less than one LD50 dose (mean dose of 0·3 mg/kg, range 0·1–0·6); five of these showed no poisoning symptoms while the other five were simply sick for 24–48 h although two exhibited hind-limb paralysis for 24–48 hr. Four other males consumed doses ranging from 1·3 to 3·5 mg/kg; two gave no poisoning

Table 4. *Bait consumption and mortality of lesser bandicoot rats given a choice between bait containing scilliroside and plain bait*

Scilliroside concentration (%)	Mortality	Mean daily bait intake/rat (g)		No. animals preferring poisoned bait	*t* test significance ($P =$)
		Poison	Plain		
0·015	15/20	2·2	6·4	4/20	0·01
0·025	9/13	1·8	3·8	7/13	N.S.
0·050	30/37	0·7	5·5	11/37	0·001

symptoms, one was sick for 1 day and the fourth convulsed for 2 days but recovered without apparent ill effects. Of the 12 female survivors, four consumed less than one LD50 dose (mean dose of 0·2 mg/kg; range 0·1–0·3); two showed no poisoning symptoms while the other two were off their food for 48 hr. Eight other females consumed one LD50 dose or more (mean = 1·2 mg/kg, range 0·5–2·8); three showed no symptoms, the remaining 5 convulsed for 24–72 h but recovered without apparent ill effects.

Palatability

On almost every trial there were individual animals that gave indications of either not feeding upon the poison bait or feeding very little, so we examined the intake of poison versus plain baits on free-choice trials at three concentrations, 0·015, 0·025 and 0·05%. Results are summarized in Table 4. Scilliroside was significantly unpalatable at both 0·015% and 0·05% concentrations, while results at 0·025% were equivocal. At the 0·015% concentration, three animals ate 0·2 g of poisoned bait or less and two survived; at 0·025% concentration two animals ate 0·2 g or less and both survived; at 0·05% concentration 14 animals ate 0·2 g or less and three survived.

DISCUSSION

The acute oral LD50 of scilliroside to lesser bandicoot rats (0·52 mg/kg for females, 0·8 mg/kg for males) indicates this species is about as susceptible to this poison as is *Rattus norvegicus*. Stoll & Renz (1941) found the oral LD50 to male rats to be 0·7 mg/kg and 0·47 mg/kg for female rats. Rothlin & Schalch (1952) reported the LD50 in 'Glaxo' strain laboratory rats to be 0·43 mg/kg in females and 2·15 in males. They state that wild grey male and female rats are 'only slightly more resistant than Glaxo rats', the only reference to the wild strain we have located. Dybing *et al.* (1952) reported the LD50 in laboratory rats of three different age groups (sexual immatures weighing 50–75 g, young mature animals of 100 to 150 g, and full grown animals weighing 200–300 g). The youngest rats were the most susceptible and showed very little difference due to sex. Fully adult males gave a LD50 of 2·5 mg/kg whereas young mature females showed the greater susceptibility of that sex, giving a LD50 of only 1·1 mg/kg. Maddock & Schoof (1970) reported a LD50 against adult male Sherman (Laboratory strain) rats

to be 0·42 mg/kg. Though Ogushi & Tokumitsu (1972) did not do a direct determination of the LD 50 on Norway rats, they concluded from feeding trials that the lethal dose in mg/kg at 0·03 and 0·05% concentration of scilliroside was 1·5 mg for males and 1·0 mg for females. There is no doubt that scilliroside is one of the most toxic rodenticides known to both the Norway rat and the lesser bandicoot rat.

The sex dependence effects of scilliroside and red squill extracts have been noted in *R. norvegicus* by other workers (Crabtree, Ward & Welch, 1939; Dieke & Richter, 1946; Barnett *et al.* 1949; Rothlin & Schalch, 1952; Dybing *et al.* 1952; Maddock & Schoof, 1970; Ogushi & Tokumitsu, 1972). Female Norway rats are 1·5–5 times more sensitive to scilliroside or red squill than are males. The cause of this sex-related effect seems determined by hormonal factors. Crabtree *et al.* (1939) found that male rats, after castration, became almost as susceptible to red squill as female rats, but regained their original resistance after administration of testosterone preparations. This sex-dependence of toxicity was not seen in other rodent species. This same sex-dependence is active in the lesser bandicoot rat, making this the only other rodent species in which this phenomenon has been observed.

The latency of scilliroside given by stomach tube to laboratory rats was reported by Rothlin & Schalch (1952) to be 4–7 h. During this period the rats were quiet and slightly comatose. This was followed by clear ataxia, spastic stiffening and an arched forward posture. Finally, spasmodic clonic-tonic extensor convulsions set in, combined with a rotatory movement of the body (rolling convulsions). In the lesser bandicoot rat, spasmodic convulsions were seen as soon as 1 h following doses of 100 mg/kg although the general observation at lower doses fit the above description by Rothlin and Schalch.

The very short duration of feeding we noted in lesser bandicoots feeding on baits containing 0·05% scilliroside is in marked contrast to the latency patterns of zinc phosphide and pyrinuron. Two animals stopped feeding abruptly with a sharp upward jerk of the head. Warning symptoms set in quickly while food, essentially, is still in the stomach.

We suspect that some animals feed upon only a small amount of scilliroside baits early in the feeding period, and then before they can return to the baits, experience warning symptoms that deter them from additional feeding. This early onset of warning symptoms (20–34 min) is an obvious disadvantage in a rodenticide. We further suspect that this accounts, in part, for the observation that varying the concentration of the active ingredient from 0·015% to 0·025% (a 17-fold increase) made little difference in mortality.

This short duration of latency, coupled with significant unpalatability to the lesser bandicoot rat, seriously impairs the efficacy of scilliroside as a poison against this species despite its highly toxic nature. However, with the paucity of relatively less hazardous acute toxicants available for use, and with the now-proved hazards of pyrinuron to humans (Prosser & Karam, 1978), we would suggest that scilliroside should continue to be evaluated, especially in a series of carefully conducted paired field trials with zinc phosphide. The optimum concentration of scilliroside for use in the field against the lesser bandicoot rat would be approximately 0·025–0·05%.

Scilliroside, even when offered at 0·025–0·05% concentration in field baits, could pose considerable hazard to children and pets and should be used with great care. We were surprised to find that the bulk of toxicological data available on this material comes basically from work done 25–30 years ago (Barnett *et al.* 1949; Dybing *et al.* 1952; and Rothlin & Schalch, 1952). Their data indicate that scilliroside possesses emetic qualities in dogs and cats but its toxicity (Rothlin & Schalch, 1952) to cats (acute oral LD50 of 6 mg/kg) and chickens (acute oral LD50 of 20–25 mg/kg) indicate it could cause problems in these species. Pigs, dogs and cats may not eat the bait (Barnett *et al.* 1949) but it should certainly be used with precautions around these species. We can find no reference to toxicity of scilliroside in primates except for this statement in Barnett *et al.* (1949): 'In two instances accidental poisoning resulted in man through inhaling the powder. The symptoms were headache, vomiting and diarrhoea within 10 hr, followed by lethargy and loss of appetite. There were no prolonged effects.'

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