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Control of the Indian mole rat with alpha-chlorohydrin: laboratory studies on bait acceptance and antifertility effects

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Summary

Toxic and antifertility effects of feeding poison baits of a toxicant-sterilant, alpha-chlorohydrin, were studied against the Indian mole rat *Bandicota bengalensis*. It was found that 0·5% alpha-chlorohydrin bait was the most palatable formulation which delivered the amount of active ingredient equal to or more than MLD (82 mg/kg) to *B. bengalensis* in a single day's feeding. The rats suffered maximum mortality with this bait concentration both in no-choice and bi-choice feeding trials. Male survivors of 0·5% and 1·0% alpha-chlorohydrin baits showed functional abnormalities of their testes as revealed by loss in testicular weight, decrease in the diameter of seminiferous tubules and thickness of seminiferous epithelium and abnormally low levels of spermatogenic cells. Effect of the poison on epididymis became apparent by the presence of epididymal lesions in caput epididymides and low levels of sperm concentration, live sperms and sperm motility in the cauda epididymal fluid. Our findings on the acceptance and toxic-cum-antifertility effects of feeding alpha-chlorohydrin baits suggest field evaluation of this poison for the management of *B. bengalensis* would be appropriate.

Key words: Rodent control, *Bandicota bengalensis*, alpha-chlorohydrin, poison bait acceptance, antifertility effects

Introduction

The Indian mole rat *Bandicota bengalensis* is a major rodent pest of South Asia (Sanchez, 1975) and appears to be the most fecund rodent species of the region as it can breed nine times in a year with average litter size of seven (Spillett, 1968; Parshad, Kaur & Guraya, 1989). Management of rodents with alpha-chlorohydrin (α-CH), which produces toxic and permanent antifertility effects, has been found to be superior than the conventional rodenticides as the former accounts for slower population rebuild up of *Rattus norvegicus* after a treatment (Andrews & Belknap, 1975, 1983; Ericsson & Daugherty, 1982). Recently, Saini & Parshad (1988) have reported that of the 15 species/strains of rodents studied so far, *B. bengalensis* seemed to be the most susceptible to the toxic and antifertility effects of α-CH. The toxic dose of alpha-chlorohydrin to *B. bengalensis* is much lower (MLD = 82 mg/kg, Saini & Parshad, 1988) than that of the *R. norvegicus* (MLD = 134 mg/kg, Kassa, 1982). No other rodent species is known to suffer mortality at less than 100 mg/kg α-CH dose.

This paper describes the results of laboratory studies on the acceptance of α-CH baits of different concentrations by *B. bengalensis* and their toxic and antifertility effects.

Materials and Methods

The Indian mole rats were trapped live from crop fields around Ludhiana, India. Mature rats (males with scrotal testes and females with perforated vagina) were used. Prior to treatment the rats were acclimatised individually in cages for 15 days and were provided with food (cracked wheat and soaked whole grams) and water *ad libitum*. The poison baits containing 0.25, 0.5 and 1.0% α-CH (supplied by Aldrich & Thomas Laboratories) were prepared by mixing α-CH concentrate with wheat flour, sugar and groundnut oil (WSG mix in the ratio of 96:2:2). WSG mix was selected because of its high acceptance by most of the rodent species in India (Bhardwaj & Khan, 1978; Ahmad & Parshad, 1985). The amount of α-CH concentrate added to obtain different bait concentrations was adjusted with equal amounts of wheat flour in the mixture. Normally equal numbers of males and females were taken for each trial. Full safety precautions were taken; general hygiene, burying of wastes and dead animals, etc.

No-choice feeding trials

After laboratory acclimatisation the singly caged rats were weighed and provided with plain WSG mix for two to three days. The rats were divided into three groups, placing six to eight rats in each group. All rats of a group were offered α-CH bait of a particular concentration in no-choice for 72 h.

Bi-choice feeding trials

One group of acclimatised rats was offered 0.5% α-CH bait while the other was provided with 1.0% α-CH bait in choice with the plain WSG mix for 72 h. The position of plain and poison bait containers was reversed every 24 h for each animal.

The treated rats were observed for 25 to 30 days to record mortality. Water was provided *ad libitum* to all rats during treatment and post-treatment periods. A record of daily food consumption was maintained for each animal. Every possible care was taken to avoid poison contamination to other laboratory material, animals and humans. The left over poison and plain baits were collected daily and buried in soil.

Study of reproductive parameters

Male rats surviving 25 to 30 days after the treatments were sacrificed using chloroform vapour. Both testes were dissected from each animal and weighed to 0.001 g. To study the effects of α-CH on the fertility of rats, live sperm count, sperm motility and sperm concentration were determined in the cauda epididymis fluid of each experimental rat by the method described by Saini & Parshad (1988).

Each testis and caput epididymis was cut into small pieces perpendicular to the long axis of the organ and fixed in alcoholic Bouins. The tissue pieces were dehydrated with graded ethanols (70, 80, 95 and 100%), cleared with benzene and embedded in paraffin wax. The testes' blocks were oriented to have most of the seminiferous tubules cut in cross section. Microtome sections were cut with thickness reading at 7 μ and keeping the speed of the block movement constant. Stretched sections on glass slides were stained with Heidenhein iron haematoxylin (Humason, 1979). Twenty five cross sections of seminiferous tubules of each animal selected at random from different slides were used for determining the diameter of seminiferous tubules, thickness of seminiferous epithelium and population of spermatogenic cells. Diameter of tubules was measured across the minor axis of their profiles. Different types of spermatogenic cells were recognised according to Perey, Clermont & Leblond (1961). Cell counts were corrected for differences in their nuclear diameters by Abercrombie's formula (Abercrombie, 1946):

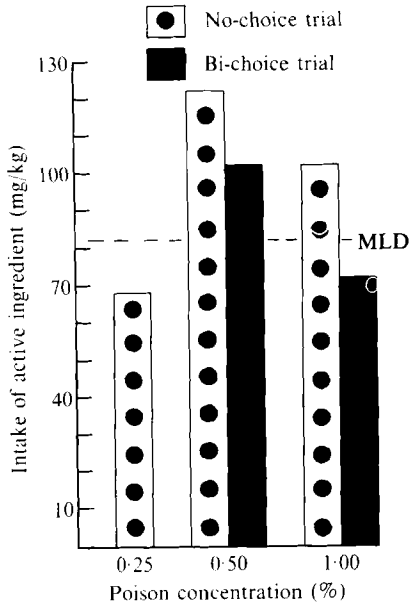

Fig. 1. First day's intake of alpha-chlorohydrin (active ingredient) in three concentrations of bait provided in no-choice and bi-choice to *Bandicota bengalensis*.

$$T = C\frac{S}{S + D}$$

where, T = true count of germ cells
C = crude count of germ cells
S = thickness of section
D = diameter of nuclei

Sections of caput epididymides were stained with haematoxylin-eosin and examined for epididymal lesions, that is presence of spermatocoeles (see Jones, 1983).

Results

No-choice feeding tests

B. bengalensis suffered maximum mortality (75%) with 0.5% α-CH bait (Table 1). Increase of α-CH in the poison bait did not enhance mortality as it affected the consumption of bait. Mortality of the animals started on day 2 and continued till the 11th day after the poison bait exposure. However, maximum deaths occurred within four to six days of poison feeding.

First day's consumption of poison bait of all the three concentrations (0.25, 0.5 and 1.0%) of α-CH was significantly less ($P < 0.05$) than the pretreatment plain bait consumption (Table 1). Consumption on the second and third day was less than that of day one and the differences were significant (ANOVA, $P < 0.01$). Daily consumption of 0.25% α-CH bait remained maximum and that of 1.0% the minimum for three days of bait exposure. However, intake of active ingredient in the first day's consumption was higher with 0.5% α-CH bait than with that of the other two concentrations of the poison (Fig. 1). Majority of the rats could consume α-CH equivalent to MLD or more in a single day's feeding from 0.5% and 1.0% baits only.

Table 1. *Efficacy of alpha-chlorohydrin bait against* B. bengalensis

Conc. (%)	Body wt (g) mean (S.E.)	Bait consumption (g)/100 g body wt; mean (S.E.) Plain Pre-treatment	Day 1 P	Day 1 CH	Day 2 P	Day 2 CH	Day 3 P	Day 3 CH	Mortality	Days to death, mean (range)
					No-Choice Tests					
0.25	200.0 (13.3)	4.9 (0.3)[a]	–	2.7 (0.5)[b]	–	1.8 (0.2)[bc]	–	1.4 (0.2)[cd]	1/6	9.0
0.50	230.9 (12.9)	6.1 (0.3)[a]	–	2.4 (0.3)[b]	–	0.9 (0.2)[c]	–	0.3 (0.1)[cd]	6/8	4.8 (4–6)
1.00	225.7 (14.2)	6.6 (0.4)[a]	–	1.0 (0.2)[b]	–	0.1 (0.1)[c]	–	0.1 (0.1)[cd]	3/6	5.7 (2–11)
					Bi-Choice Tests					
0.50	223.4 (13.3)	4.7 (0.4)	1.8 (0.3)	2.0 (0.2)[NS]	0.6 (0.2)	0.5 (0.1)[NS]	0.5 (0.2)	0.5 (0.2)[NS]	5/10	5.6 (3–8)
1.00	209.8 (10.5)	6.8 (0.3)	1.7 (0.1)	0.7 (0.1)**	1.1 (0.2)	0.3 (0.1)**	1.1 (0.4)	0.1 (0.1)*	1/6	5.0

a,b,c,d are the ranks assigned on the basis of critical difference between the means.
* = $P < 0.05$; ** = $P < 0.01$; – significant differences by Student's t-test between poison and plain bait consumption of the same day.
NS = non-significant difference ($P > 0.05$).
P = plain bait.
CH = α-chlorohydrin bait.

Table 2. *Effect of alpha-chlorohydrin bait on the reproductive parameters of male* B. bengalensis

Poison bait concentration (%)	Male rats (survived/ treated)	Testes wt. (mg)/ 100 g body wt. mean (S.E.)	Sperm concentration (10^6/ml) mean (S.E.)	Live sperm count (%) mean (S.E.)	Sperm motility (%) mean (S.E.)	Rats with abnormal sperm motility (<30%)
Control	6/6	491.8 (16.4)	228.1 (9.1)	92.1 (3.6)	87.9 (8.5)	0
			No-Choice Tests			
0.25	3/3	473.4 (102.8)	212.8 (50.4)[b]	38.1 (7.2)[a]	31.8 (10.5)[a]	2
0.50	1/4	338.8	163.0	43.0	23.0	1
1.00	2/3	198.9 (38.5)[a]	36.5 (35.5)[a]	10.5 (10.5)[a]	8.0 (8.0)[a]	2
			Bi-Choice Tests			
0.50	3/5	191.8 (53.2)[a]	0	0	0	3
1.00	2/3	407.1 (39.7)	58.1 (17.0)[a]	36.8 (17.3)[a]	13.6 (6.2)[a]	2

a = $P < 0.01$; b = $P < 0.05$ — significant differences by t-test between untreated and treatment.

Table 3. *Effect of alpha-chlorohydrin baits on the testicular and epididymal functioning in* B. bengalensis

Poison bait concentration (%)	Survived male rats	Diameter of seminiferous tubule (μ) mean (S.E.)	Thickness of seminiferous epithelium (μ) mean (S.E.)	Spermatogenic cells/cross section of the seminiferous tubule, mean (S.E.)			Rats with epididymal lesions
				Spermatogonia	Spermatocytes	Spermatid (round)	
Control	6	188.1 (4.1)	64.4 (2.1)	24.6 (1.7)	31.9 (1.8)	74.6 (4.3)	0
			No-Choice Tests				
0.25	3	168.3 (6.7)[b]	56.2 (3.1)[b]	22.0 (1.9)	30.9 (2.8)	68.8 (4.1)	0
0.50	1	129.1 (10.1)[a]	41.3 (5.6)[a]	9.8 (2.1)[a]	21.4 (5.8)[a]	24.2 (4.1)[a]	1
1.00	2	102.3 (7.3)[a]	34.9 (3.7)[a]	13.0 (4.1)[a]	18.1 (4.3)[a]	18.2 (6.6)[a]	2
			Bi-Choice Tests				
0.50	3	99.8 (7.2)[a]	32.6 (6.0)[a]	4.6 (2.1)[a]	13.4 (3.3)[a]	0	3
1.00	2	153.9 (5.7)[a]	51.0 (2.0)[a]	15.8 (3.1)[a]	22.1 (5.1)[a]	34.2 (4.6)[a]	2

a = $P < 0.01$; b = $P < 0.05$ - significant differences by t test between untreated and treatment.

Bi-choice feeding tests

Food consumption data revealed that the acceptance of 1.0% α-CH bait was significantly less than 0.5% α-CH bait when offered separately with plain alternative in bi-choice (Table 1). Fifty percent mortality of *B. bengalensis* was achieved with 0.5% α-CH bait, whereas, only one out of six experimental rats died when 1.0% α-CH bait was offered. The maximum number of rats died within one week of their exposure to poison baits.

No difference was observed in the consumption of 0.5% α-CH and the alternative plain bait ($P > 0.05$), whereas, the consumption of 1.0% α-CH bait was significantly less ($P > 0.05$) than the plain alternative. Combined consumption of plain and 0.5% α-CH baits on the first day of bi-choice trial (3.8 g/100 g body weight) was similar to the pretreatment plain bait consumption (4.7 g/100 g body weight) but the differences became significant ($P < 0.01$) for the second and third day of exposure when total daily consumption was recorded to be very low (1.1 and 1.0 g/100 g body weight respectively; Table 1). In the other trial, combined consumption of plain and 1.0% α-CH bait remained less than the pretreatment consumption for all three days of bi-choice feeding.

Reproductive parameters

Testicular weights of the surviving male *B. bengalensis* from no-choice trial with 1.0% and bi-choice trial with 0.5% α-CH bait were recorded to be lower (about 200 mg/100 g body weight) than that of the control group (492 mg/100 g body weight) and the differences were significant ($P < 0.01$; Table 2). The diameter of seminiferous tubules and thickness of seminiferous epithelium decreased significantly ($P < 0.05$) at all concentrations of α-CH bait in no-choice and bi-choice trials and the decrease in these two parameters was positively correlated ($r = 0.7425$, D.F. 273 $P < 0.01$; Table 3). Except for the rats fed with 0.25% α-CH bait in no-choice all individuals of other trials had low population of spermatogenic cells (spermatogonia, spermatocytes and spermatids) in their testes as compared to that of the control animals.

Ingestion of 0.5 and 1.0% α-CH baits caused conspicuous functional abnormalities in the epididymides of *B. bengalensis*. Compared to that of the control, the cauda epididymides of treated rats showed a significant decrease in sperm concentration, live sperms and sperm motility. Abnormal level of sperm motility (< 30%) was observed in almost all the surviving male rats. However, aspermia (no sperm in the cauda epididymis fluid) developed only in the survivors of 0.5% α-CH bait in the bi-choice feeding trial. Rats did not suffer any damage to their epididymides with 0.25% α-CH bait, whereas, the other two concentrations of α-CH bait offered in no-choice and bi-choice affected the caput epididymides and led to the development of spermatocoeles.

Discussion

Bait acceptance and toxicity

Our studies have revealed that 0.5% α-CH bait is a better formulation for treating *B. bengalensis* because of its good acceptance and effectiveness. Though the first day's intake of 0.25% α-CH bait was higher than that of the other two formulations, the consumed amount was insufficient to produce toxic and sterility effects. In contrast, the rats ate only very small amounts of 1.0% α-CH bait.

Daily intake of food decreased during the trials and, generally, the intake of poison bait was less than the plain alternative except for 0.5% α-CH bait in bi-choice where the two alternative foods were eaten almost equally. Earlier, Meehan & Hum (1979) had reported poor palatability of α-CH baits by Wistar rats (a laboratory strain of *R. norvegicus*) and observed

that bait consumption decreased with increase in poison concentration. For wild-caught *R. norvegicus* 1.0% α-CH bait was found to be better in effectiveness (Kassa & Jackson, 1984). The present studies have shown that 0.5% concentration of α-CH in bait is sufficient to deliver the amount of active ingredient equivalent to or more than MLD value (82 mg/kg) to *B. bengalensis* in a single day's feeding. However, none of the *R. norvegicus* could consume poison equivalent to the MLD with 0.5% α-CH bait (Kassa & Jackson, 1984). This difference in the response of two species can be related to the relative susceptibility of *B. bengalensis* (MLD = 82 mg/kg, Saini & Parshad, 1988) and *R. norvegicus* (MLD = 134 mg/kg, Kassa, 1982) to α-CH.

Compared to 100% mortality of *B. bengalensis* with single oral administration of 100 mg/kg dose of α-CH (Saini & Parshad, 1988) only about 50% mortality occurred when the rats ingested equivalent amounts of the chemical during 24 h of feeding the 0.5% and 1.0% baits. This might have happened because the intake of active ingredient during 24 h of feeding most probably occurred in more than one instalment and, therefore, its effectiveness cannot be compared with the orally administered dose in a single instalment as α-CH has no cumulative toxicity and gets regularly detoxified and eliminated from the body through urine, faeces and breath (Ericsson, Downing, Marsh & Howard, 1971). Meehan & Hum (1979) had also indicated that mortality of rats correlated with greatest amount of active ingredient eaten in a single day.

Antifertility effects

Functional abnormality of the testes, pertaining to their loss of weight and decreased sperm production, was evident in the survived individuals of 0.5% α-CH bait in no-choice and bi-choice trials and of 1.0% bait in no-choice because a majority of the rats in these three trials consumed poison equivalent to MLD or more than that (Fig. 1). Previous studies have shown that following a high dose of α-CH the testes become enlarged and increase in weight for approximately five days after which a constant decrease in testicular weight becomes evident due to a pressure mediated necrosis caused by the development of spermatocoeles in the caput epididymides (see Jones, 1983). This pathological lesion is responsible for preventing the passage of immature testicular sperms to the epididymal tract (Cooper & Jackson, 1973; Hoffer, Hamilton & Fawcett, 1973; Kassa & Jackson, 1984). As a consequence, the sperm number gets reduced in the cauda epididymis and in the ejaculate. Similarly, in our studies the sperm concentration in cauda epididymides of all the treated groups was significantly less than that of the control group. Except for the individuals offered with 0.25% α-CH bait in no-choice, all other survivors had developed epididymal lesions and were recorded for abnormally low levels of sperm motility (< 30%). Direct effect of α-CH on the motility and fertility of rat epididymal sperms *in vivo* has been confirmed (Chulavatnatol, Eksittikul & Wongkam, 1981; Tsang, Lee & Wong, 1981; Paz & Homonnai, 1982). Numerous studies have suggested glyceraldehyde– 3 –phosphate dehydrogenase, an enzyme in the glycolytic pathway of sperms, is inhibited so that ATP levels adequate for sperm motility and consequently for successful fertilisation cannot be maintained (see Jones, 1978).

Shrinkage of seminiferous tubules, revealed by the decrease in diameter and thickness of seminiferous epithelium, along with marked low levels of spermatogenic cells tend to educe the fact that a certain level of testicular necrosis had occurred. The effect was more pronounced in the survivors of 0.5% α-CH bait in bi-choice whose survivors had practically no spermatid as observed in testicular sections.

Kassa & Jackson (1981) had observed that sterile (α-CH treated) *R. norvegicus* males successfully induced vaginal plugs in the receptive females, thus indicating no impairment of libido. Other studies have also shown that α-CH does not affect libido of the males (see Jones, 1983). Male survivors of *B. bengalensis* offered with α-CH bait can also be expected to mate

with receptive females and, consequently, contribute in slower population rebuild up as has been demonstrated in preliminary field trials with *R. norvegicus* (Ericsson & Daugherty, 1982; Andrews & Belknap, 1983). Moreover, we have observed the males and females of *B. bengalensis* to be equally susceptible to the toxic effects of α-CH.

So, the present studies tend to indicate the potential of α-CH for the control of *B. bengalensis* as its 0.5% bait has been found to be palatable to the rats. The rats ingested the active ingredient equal to MLD (82 mg/kg) or more than that within 24 h of feeding the α-CH bait, suffered mortality of both sexes and above all the survived males were observed to be practically sterile. In the light of these findings, field efficacy of α-CH should be evaluated for the control of *B. bengalensis*.

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