USE OF THE MALE CHEMOSTERILANT: α-CHLOROHYDRIN IN THE CONTROL OF HOUSE RATS, *Rattus rattus*

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Abstract

The male antifertility compound: α-chlorohydrin (ACH) was tested for the control of Roof Rats, *Rattus rattus*. The compound was provided to rats as 1% ACH/crushed maize formulation for three successive days. The concentration and the period of application used were effective in producing permanent sterility in males. Two of the twelve tested males (16.6%) were died after two days. They ingested average daily amounts of ACH of reached 207.1 and 346.6 mg/kg body weight, respectively. The average daily amounts of ACH ingested by the ten surviving males over three successive days, ranged between 81.8 and 266.6 mg/kg. The microscopic examination of the testes and epididymides of rats sacrificed 7, 35 and 60 days after the application of ACH indicates that these organs were severely damaged and the process of spermatogenesis was greatly reduced. Most seminiferous tubules of examined testes were free of spermatozoa. In rats sacrificed after 60 days, most seminiferous tubules were atrophied and shrunk with extensive exfoliation of germ cells into the lumina of tubules. Epididymides of treated rats showed interstitial edema, and immature germ cells in the lumina of their ducts.

INTRODUCTION

The inefficiency of lethal methods of pest control has forced pest managers to search for non-lethal alternatives. One of these alternatives is the use of antifertility compounds. The final goal of such compounds is to reduce the size of pest populations, by suppressing reproduction, rather than by increasing mortality. This approach is, therefore, more humane and morally acceptable than conventional pest control methods. Moreover, antifertility compounds could be used in areas where the use of lethal techniques is restricted such as in human habitations. The effectiveness of chemosterilants is based on the concept that sterile individuals in a population exert much greater control than if the same number of fertile individuals were removed (Howard, 1967). This is due to the fact that sterile individuals continue to compete for territory, food, social order, and sexual partners. Females mated with sterile males

exhibit pseudopregnancy which limits coitus with fertile males, and with time, their reproductive capacity (Ericsson, 1970).

Male chemosterilants, as a group of antifertility compounds, have been evaluated for use in rodent control (Marsh and Howard, 1970). Only the non-steroidal ACH possesses most of the attributes of an ideal male antifertility agent and appears to have practical application in rodent control. Low doses of this compound cause reversible antifertility response since it interferes with the function of spermatozoa (Coppola, 1969; Ericsson and Baker, 1970), while high doses can lead to prolonged or permanent infertility (Jones, 1983). At still higher doses, the compound is toxic (Ericsson and Baker, 1970). ACH is effective in various mammals such as rats, rams, boars, guinea pigs, hamsters, and rhesus monkeys (Jones, 1983).

The present work aims to evaluate the effect of 1% ACH formulation applied for three successive days in producing permanent sterility in male roof rats. The ACH formulation acceptance by caged roof rats as well as the toxicity and antifertility effects of this formulation, as indicated by the histological examination of the testes and caudapididymides, are investigated.

MATERIALS AND METHODS

ACH as a male chemosterilant

ACH (3-chloro-1,2-propanediol) used in the present study is a product of Sigma-Adrich Company. It has a concentration of 98%, anda density of 1.322g/mLat 25◦ C.

Test rats

A total of twelve males of commensal Roof Rats were used in the present work. Rats were individually caged in wire cages (50 x 30 x 20 cm), acclimatized for at least 2 weeks. Food (bread) and water were provided *ad libitum.* Inactive (unhealthy) rats were excluded and replaced by healthy ones. Cage tests were conducted on these males to evaluate the acceptance of 1% ACH formulation, and the antifertility effect of this compound.

Preparation of plain food and ACH formulation

The plain food was prepared by mixing crushed maize, vegetable oil, and powdered sugar in the following ratios: 90:5:5, respectively. The 1% ACH formulation was prepared by mixing ACH (0.98) with the above mixture in a ratio of 1:97 w/w.

Pre-treatment non-choice feeding tests

Forty grams of plain food, placed in a small dish, were provided daily to each of twelve individually caged male rats for three successive days. Water was provided *ad libitum*. The daily amounts of plain food removed by individual rats were estimated.

1% ACH formulation non-choice feeding tests

The above test was repeated using the 1% ACH formulation. Water was provided *ad libitum*. The daily amounts of the toxic formulation removed by individual male rats were estimated according to the guidelines recommended by Mathys (1975).The acceptability of the poison bait was calculated using the equation of Mason *et al.* (1989):

$$\text{Acceptability (\%)} = \frac{\text{Average daily consumption of 1\% ACH formulation (g)}}{\text{Total average daily consumption of (1\% ACH formulation+ plain food) (g)}} \text{ X } 100$$

Post-treatment feeding and observation

Forty grams of plain food, placed in a small dish**,** was provided daily to each of the individually caged treated male rats for 7 successive days. Water was provided *ad libitum*. The daily amounts of plain food removed by individual rats were estimated, and rats were observed for mortalities.

Test-groups and microscopic preparations

The treated rats were divided into 3 test-groups. Plain food and water were provided *ad. libitum.* The first and second groups consisted of 4 rats each while the third group consisted of 2 rats. Males of the first group were sacrificed 7 days after the stop of ACH application, those of the second and third groups were sacrificed 35 days and 60 days, respectively, after the stop of application. Rats of each group were sacrificed by an overdose of chloroform. The testes as well as the epididymides were dissected out for gross examination and microscopic preparation. Routine hematoxylin-eosin preparations were made of the testes and epididymides of each male rat. The preparations were examined by the light microscope for identifying the presence of spermatozoa and for histological changes.

RESULTS

I. Consumption of plain food and 1% ACH formulation

The plain food as well as the 1% ACH formulation were offered to twelve individually caged male roof rats for three successive days in non-choice feeding tests. The results are presented in table (1)

The individual daily consumption of plain food and 1% ACH formulation ranged between 7.6 to 16.3 g and 1.4 to 5.2 g, respectively. The ingested daily amounts of ACH expressed as mg/kg body weight ranged between 81.8 to 266.6 mg/kg. Two of the twelve tested males died after two days. They ingested average daily amounts of ACH reached 207.1 and 346.6 mg/kg, respectively.

II. Effect of ACH on the testes and epididymides of male rats

Microscopic preparations of the testes, caput epididymides and caudaepididymides of rats treated with ACH were examined for the presence of spermatozoa and for histopathological changes produced by this antifertility compouned. It is clearly evident from the examination of these preparations that the daily amounts of ACH ingested by individual rats have profoundly affected spermatogenesis and caused permanent sterility to male roof rats of the present study. None of the examined preparations showed mature spermatozoa.

i. Effect on testes

The ingestion of an average individual daily amount of 169.4 mg/kg (81.8-266.6 mg/kg) of ACH, for three successive days, resulted in permanent sterility in treated males since it caused a severe damage to their testes. Marked atrophy of seminiferous tubules was observed in treated rats. Maturation arrest of spermatogenic cells were evident in the testes of rats sacrificed after 7 days. Dislodged and sloughed off germ cells into tubules, and decreased cellularity of germ cells were observed in the testes of rats sacrificed 35 and 60 days after the application of the compound, respectively. Other histopathological effects of the compound are represented by marked interstitial as well as intratubular edema in the testes of rats sacrificed 35 and 60 days after the application of the compound (Plate 1a,d,g).

Table 1. Average daily consumption of ACH by male roof rats in non-choice feeding tests. Mean is followed by S.D. and the range (in parentheses).

Tested rats	Days after which rats were sacrificed	Body weight (g)	Average daily consumption				Average ACH ingested by individual rats of each group (mg/kg)	Acceptability (%)
			Plain food (g)	1% ACH formulation (g)	ACH (mg/indv.)	ACH (mg/k)		
1	Died after 2 days	140	9.1	2.9	29	207.1	Excluded from the study	19.86
2		150	7.7	5.2	52	346.6		
3	7 days (First group)	150	10.7	3.9	38	260	209.2±61.7 (147.8-264.7)	
4		230	9.2	3.4	33	147.8		
5		170	16.3	4.5	45	264.7		
6		140	15.3	2.3	23	164.2		
7	35 days (Second group)	220	13.3	1.8	17	81.8	147.8±86.0 (81.8-266.6)	
8		230	9.5	1.9	19	82.6		
9		120	13.3	3.2	31	266.6		
10		100	7.6	1.6	16	160		
11	60 days (Third group)	100	11.5	1.4	14	140	133.3±10.1 (126.6-140)	
12		150	13.7	1.9	19	126.6		
Average		158.3±46.0 (100-230)	11.4±2.9 (7.6-16.3)	2.8±1.2 (1.41-5.2)	28.3±12.3 (14-52)	187.2±82.3 (81.8-346.6)		

ii. Effect on epididymides

The examination of epididymides of treated males revealed the presence of focal areas of haemorrhage, with some tubules obstructed with fibrous connective tissues and inflammatory exudates (7 days). Interstitial edema was observed in the epididymides of the three groups of treated rats. The presence of immature germ cells was observed in the lumina of caput epididymides of all groups, and the lumen of cauda epididymis of the third group (Plate 1b,c,e,f,h,i).

DISCUSSION

Non-steroidal chemicals that affect male fertility have been known for over 50 years. Of these chemicals, only ACH possesses most of the attributes of an ideal male contraceptive (Jones, 1983). According to this author, ACH produces an immediate and continuous antifertility response, does not interfere with libido, not toxic at low doses, and has species-specific action. High doses ACH produce irreversible effects on the epididymis and induce sterility. A single high dose produces a characteristic pathological lesion in the initial segment of the epididymis (Hoffer *et al.*, 1973). Low doses of ACH produce a reversible reduction in fertility after 3-6 days with fertility recovering in the treated animal approximately 5-11 days after cessation of ACH administration (Tsunoda and Chang, 1976). ACH is a well known food contaminant that is found in various food products such as soy sauce, bread, biscuits, cheese, refined oil, as well as in drinking water treated with epichlorohydrin resins (Zhang *et. al.*, 2012).

In the present study, 1% ACH formulation was offered to caged males in non-choice feeding tests for three successive days. The average daily amounts ingested by males ranged between 81.8-266.6 mg/kg. These amounts proved to produce permanent sterility in males as indicated by the histological examination of the testes and caudaepididymides. Two of the tested males died after ingesting average daily amounts of 207.1 and 346.6 mg/kg for two days, and their sexual organs were not processed for histological examination. Antifertility responses were observed in wild male *R. rattus* treated with much lower amounts of ACH (10 mg/kg body weight) given daily through intragastric intubation, but for extended periods of 5, 15, and 45 days. The responses included reduced seminiferous tubular areas and a steady decline in the percentage of spermatozoa in treated males, and lower number of implantation sites in females paired with treated males (Madhu*et al.*, 2011). The amounts required to produce permanent sterility in Norway rats ranged between 90-100 mg/kg in a single oral dose (Ericsson, 1982). A dose of ≥ 100 mg/kg administered by oral intubation produced epididymal lesions with the formation of spermatocele and sperm granuloma in these rats (Kasa and

Jackson, 1981).In their experimental work to induce sterility in males, various authors treated animals with different doses of ACH and in different ways. The lowest oral doses were 5-10 mg/kg used for few days (Jones, 1983). An injection of 40 mg/kg for 20 days (Samojlik and Chang, 1970), a single intraperitoneal dose of 75mg/kg (Cooper and Jackson, 1973), and a subcutaneous dose of 90 mg/kg (Brown and White, 1978) were used with albino rats. Single oral doses of 140 mg/kg (Hoffer *et. al.*, 1973) and 90-100 mg/kg (Ericsson, 1982) were used in rats. Temporary effects of ACH were produced in Norway rats by a single oral dose of 15-20 mg/kg (Ericsson, 1982). These doses are considerably lower than the doses ingested by roof rats in the present study. Higher doses are possibly required to produce infertility effects if the compound is administered with a bait rather than by oral intubation (Kasa and Jackson, 1981). However, high doses of 200-300 mg/kg, that are comparable to doses ingested by rats in the present study, were given by oral intubation to Indian mole rats by Manjet and Parshad (1988).

Several studies were carried out to explain the mechanism of ACH effect on the fertility of treated males. Histopathological examination of the testes of albino rats treated with ACH reveals a total inhibition of spermiogenesis as indicated by the degeneration and disappearance of spermiogonia from the tubules, with a proliferation of the epithelial cells of the caudaepididymal ducts (Samojlik and Chang, 1970). The compound causes a sloughing of the epididymal epithelium that leads to the obstruction of the epididymal tract (Hoffer *et al.*, 1973). A single intraperitoneal injection of ACH results in bilateral retention cysts or spermatocele of the caput epididymis 5 to 7 days after injection (Cooper and Jackson, 1973). ACH is thought to cause local ischaemia, with resultant epithelial desquamation which blocks the caput epididymis, followed by the formation of spermatoceles, sperm granulomata, and fibrosis (Ericsson, 1970). According to the same author, the immediate consequences of the lesion are sperm blockage in ductuliefferentes and testicular swelling. Fluid accumulation in the testis causes pressure degeneration of the germinal epithelium. Testes become small in size due to the presence of many non-functional seminiferous tubules resulting in prolonged or permanent sterility.

Many studies have dealt with the effect of ACH on spermatozoa, and suggest that the antifertility effect of ACH in rats is primarily exerted by a direct action on spermatozoa (Black *et al.*, 1975, Jones, 1978). It causes an inhibition of the motility of spermatozoa, partly due to alkalation of the spermatozoa amino acid cysteine (Kalla and Bansal, 1977). The results of other studies indicate that the reduced motility of spermatozoa is due to an inhibitory action of the metabolites of ACH on the enzymes involved in spermatozoa glycolysis (Gil and Guraya, 1980; Jones, 1983). The most susceptible enzyme appears to be glyceraldehyde-3-phosphate-dehydrogenase

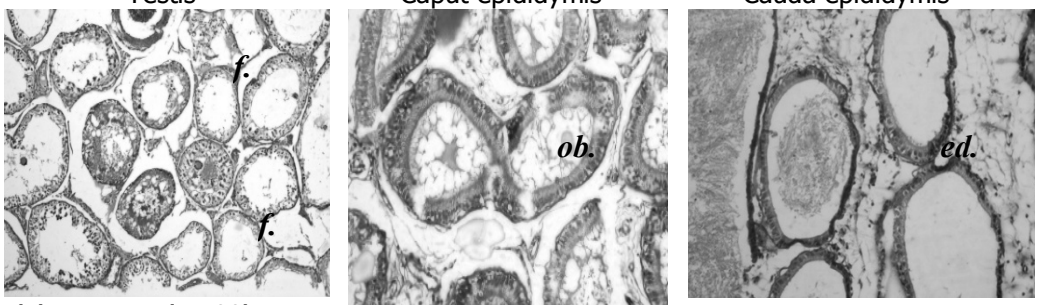
(Dickinson *et al.*, 1977; Jelks and Miller, 2001). Ultrastructural studies have indicated the occurrence of severe morphological abnormalities in spermatozoa, including deglutination of the acrosomal part, loss of head capsules, and fragmentation of tail fibrils (Madhu*et al.*, 2011). Reduction in epididymal sperm velocity was earlier observed in rats treated with ACH (Ericsson and Baker, 1970). The compound rendered them incapable of fertilization after a few days without causing any visible changes in their morphology (Jones, 1983). A reduction in epididymal sperm velocity, associated with both a delay and failure of fertilization *in vivo*, was also recorded in hamsters treated with ACH (Valerie *et al.*, 1997). However, Brown and White (1978) recorded a disruption of the morphology as well as motility of epididymal sperms in rats after a subcutaneous injection of ACH.

CONCLUSION

It is concluded from the results of the present study that ACH could be used as a management tool against roof rats. It causes permanent sterility in males when consumed in average daily amounts of 81.8-266.6 mg/kg for three successive days. Since it does not affect their sexual behavior, permanently sterilized males can compete for mates thus reduce fertile mating. Further studies under semi-field as well as under field conditions are required. It is recommended that ACH, as a male chemosterilant, could be used in the control of roof rats in an integrated pest management approach.

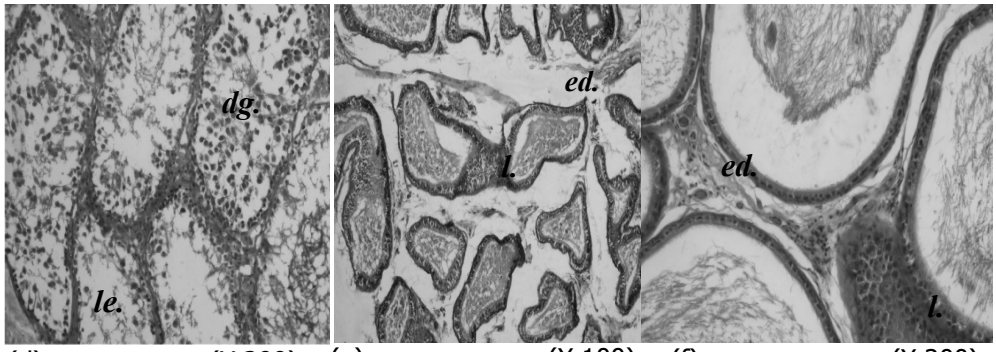
Plate 1

Testis | Caput epididymis | Cauda epididymis



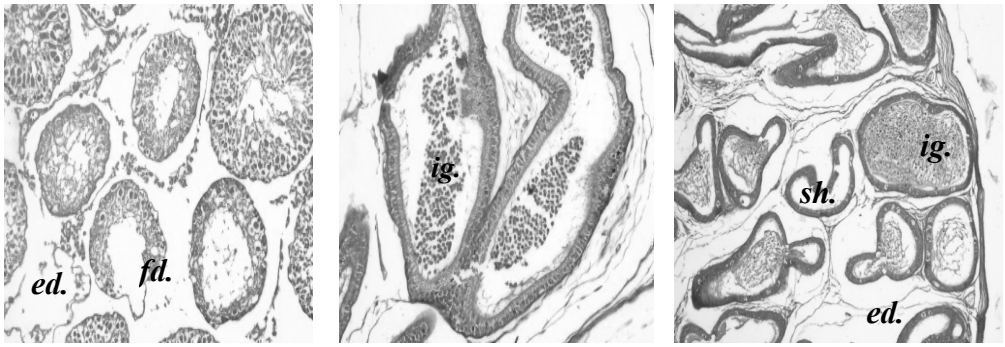
(a) (X 100) (b) (X 200) **(c)** (X 200)

First group



(d) (X 200) (e) (X 100) (f) (X 200)

Second group



(g) (X 400) (h) (X 200) (i) (X 200)

Third group

Photomicrographs (H & E) of testes, caput epididymis, and caudaepididymidis of rats sacrificed 7 days (first group), 35 days (second group), and 60 days (third group) after the application of ACH. *dg.*dislodged and sloughed germ cells, *ed.* edema, *f.* free of spermatozoa, *fd.*focal germ cell depletion, *ig.*immature germ cells, *l.* focal hyperplastic lesion of epithelial lining, *le.*, complete loss of epithelium, *ob.* obstructed, *sh.* shrunk and atrophied duct.

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استخدام مادة ألفا-كلوروهيدرين المسببة لعقم الذكور فى مكافحة الجرذ المنزلى *راتس راتس*

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يهدف هذا البحث إلى اختبار كفاءة جرعات مناسبة من مادة "ألفا-كلوروهيدرين" فى إحداث عقم دائم فى ذكور الجرذ المنزلى. وتعتبر هذه المادة أفضل المواد الكيميائية المسببة للعقم فى الذكور حيث أنها سريعة المفعول، ولا تؤثر على السلوك الجنسى للذكور، وهى غير سامة عند استخدامها بتركيزات منخفضة، كما أنها تستهدف الآفات المراد التخلص منها دون غيرها من الحيوانات الأخرى. ولقد أوضحت النتائج فعالية هذه المادة في إحداث عقم دائم فى الذكور التى تناولتها عندما تم تقديمها بتركيز ١% فى خليط يتكون من جريش الذرة والسكر والزيت النباتى لمدة ثلاثة أيام متوالية، وكانت نسبة استساغة هذا الخليط ١٩.٩%. ولقد تسببت هذه المادة فى إحداث وفيات بين الجرذان وصلت إلى ١٦.٦% بعد يومين من تقديمها بالتركيز المذكور. وأوضح الفحص الميكروسكوبي لقطاعات من الخصية والبربخ لذكور الجرذان التي تم الإبقاء عليها ٧ و ٣٥ و ٦٠ يوما بعد انتهاء تقديم هذه المادة توقف عملية تكوين الحيوانات المنوية بدرجة كبيرة بالإضافة إلى حدوث تأثيرات مرضية فى أنسجة كل من هذه الأعضاء.