Indian J Physiol Pharmacol 1990; 34(3): 215-17

SHORT COMMUNICATION

BHC INDUCED TESTICULAR IMPAIRMENTS IN RATS

A. ROY CHOWDHURY AND A. K. GAUTAM

National Institute of Occupational Health,
Meghani Nagar,
Ahmedabad - 380 006

(Received on February 26, 1990)

Abstract : Benzene hexachloride (BHC) was fed to mature male rats weighing 160 g at dosages of 3 and 6 mg/kg body weight over a period of 180 days. Significant decrease in testicular weight and degeneration of seminiferous tubules with deformed spermatogenic cells were noted at a dose of 6 mg/kg BHC. Marked increase in BHC residue in testis revealed that the drug was able to cross blood-testis barrier.

Key words : BHC spermatogenic cells degeneration

INTRODUCTION

Benzene hexachloride (BHC) is a common and widely used pesticide in India. The technical grade BHC is a mixture of several stereo isomers showing low acute toxicity but high chronic toxicity to animals mainly due to accumulation and slow degradation of its ß-isomer in animal tissues (1, 2). Various reports have shown that BHC causes several systemic disorders in liver and reproductive organs in workers and animals exposed to the drug. Teratogenic effects of the drug have also been reported (3-5). Therefore, the present study is an attempt to observe in rats the effect of orally fed BHC on spermatogenesis and residual analysis of BHC in testicular tissues.

METHOD

Adult male albino rats of Charles Foster strain, weighing 160±5 gm were acclimated to laboratory conditions, divided into three equal groups and maintained on standard diet and water *ad libitum*. Group I (Gr. I) served as control. Rats of Group II and III (Gr. II, III) were fed technical grade BHC (Hindustan Insecticides, New Delhi) as suspension in gum acasia by intubation at dosages of 3.00 and 6.00 mg/kg body weight respectively (0.6% & 1.2% of LD_{50}; 500 mg/kg orally) over a period of 180 days, controls were given only gum acasia solution for the same period. Body weight was recorded twice a week and animals were observed for any overt sign of toxicity during the experimental period. After 180 days of treatment, blood was collected from the retro-orbital venous plexus and serum was seperated by centrifugation. Serum and tissue BHC residuces were extracted with the use of hexane (HPLC-Grade) and estimated by GLC, Model 3920 B, Perkin Elmer. The samples were run through column (Column composition is 5% OV-17) at 225°C. The flow of sample, containing argon and methane-5%, was maintained at 60 ml/min (6). The ECD (Electron Captur Detector) was used in this method.

The animals were sacrificed by decapitation and testis were quickly dissected out and weighed. Testis from each group were fixed in Bouin's fluid, 5 μm thick paraffin sections were stained with hematoxylin and periodic acid Schiff's reagent (PAS) for the microscopical examination. Quantitative assay of spermatogenesis was carried out at 1600 fold magnification (7). Diameter of seminiferous tubules and Leydig Cell nucleus was determined by Ocular micrometer at 160 and 640 magnification respectively. Leydig cell nuclear population was determined at 640 magnification (8).

The mean values of all parameters were subjected to statistical analysis using Student 't' test.

RESULTS AND DISCUSSION

Significant decrease in body and testicular weights in Gr. II and III indicated growth retardation in BHC fed rats over a period of 180 days (Table I). Moreover, the elevated BHC residue in serum and testis in the same groups indicated the toxic manifestation in treated rats (Table II). Earlier experimental studies indicated that lindane, an important constituent of BHC, caused degenerative changes in rat testis (9,10). Significant decrement in seminiferous tubular and Leydig cell nuclear diameter in both the treatd groups suggest that BHC affects both gametogenic and steroidogenic functions of the testis (Table II). In Gr. II, detachment of the germinal cells from peritubular membrane and atrophy of Leydig cells along with intertubular oedema were observed in comparison to control (Fig. 1a, b). Complete degeneration of

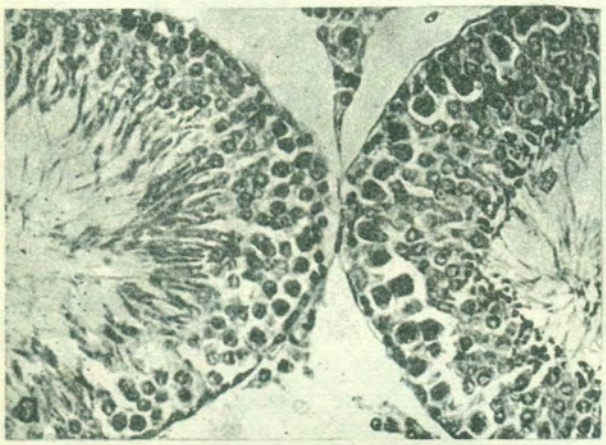
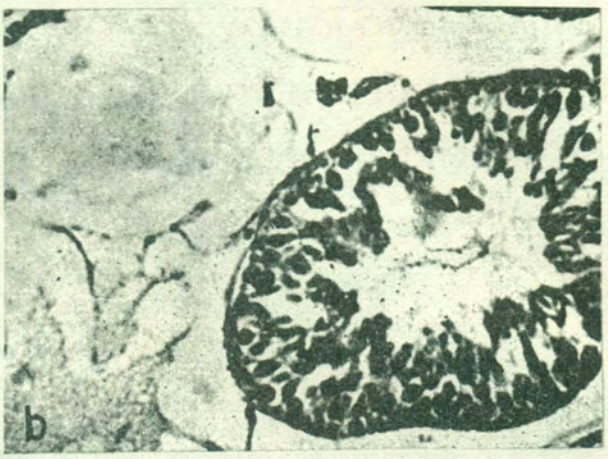
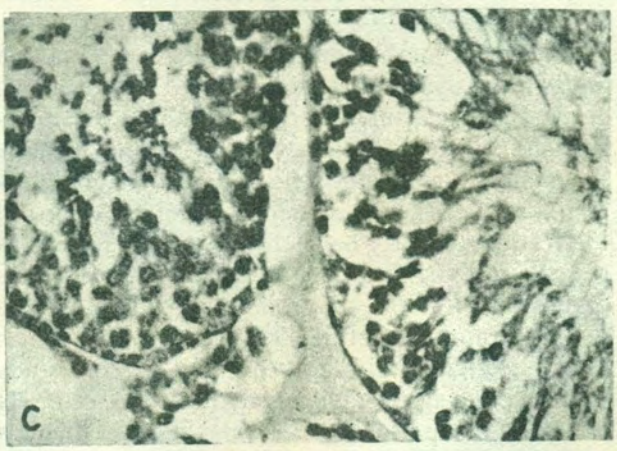


Fig. 1: (a) Control rat testes showing normal pattern of spermatogenic and Leydig cells × 640.



(b) Showing degenerated seminiferous tubules and Leydig cells after BHC feeding to rats at the dose of 3 mg/kg for 180 days, × 640.



(c) Showing complete degeneration of gametogenic cell and inter tubular oedema in rats after feeding 6 mg/kg BHC for 180 days × 640.

TABLE I : Effect of different dosage of BHC orally on body and testicular weight of rats, In each case number of observations are 10 in number. Mean±S.E.

Group	*Body weight (g)*		*% gain in body weight (g%)*	*Testicular weight (Absolute) (g)*
	Initial	*Final*		
Control (Gr. I)	168.67±5.46	390.0±2.91	131.22	1.42±0.05
3 mg/kg (Gr. II)	164.70±1.93	345.2±2.52*	109.59**	1.23±0.04*
6 mg/kg (Gr. III)	166.00±2.58	353.8±6.95*	112.96**	1.13±0.04**

* P<0.05, ** P<0.001, Compared as Gr. I Vs Gr. II, Gr. I Vs Gr. III

TABLE II : Effect of oral feed BHC at dosages of 3 mg/kg & 6 mg/kg on the spermatogenic cell counts and total residual level of BHC in serum and testicular tissues. In each case number of observations are in parenthesis. Mean±S.E.

Group	*Seminiferous tubular diameter* (μm) (10)	*Spermatogenic cell/ 8.0 Sertoli cells (10)* Count				*Leydig cell nuclear* diameter (μm) (10)	*Leyding cell nuclear* population sq/m. (10)	*BHC residue*	
		Spg	Rspc	Pachy	7-Sptd			Serum (5) (μg/ml)	Testis (5) (μg/g)
Control	311.0	4.85	18.72	27.69	23.10	6.60	10.30	0.2681	0.1597
(Gr. I)	±	±	±	±	±	±	±	±	±
	4.03	0.25	0.24	0.30	0.49	0.22	0.37	0.021	0.014
3 mg/kg	277.5	3.49	13.74	13.10	6.57	5.8	8.10	0.3193	1.892
(Gr. II)	±	±	±	±	±	±	±	±	±
	2.64*	0.09**	0.37	0.63**	0.11	0.25	0.31**	0.012*	0.252**
6 mg/kg	265.5	0.45	3.28	4.25	2.24	5.30	6.40	0.393	1.939
(Gr. III)	±	±	±	±	±	±	±	±	±
	3.55**	0.022**	0.15**	0.08**	0.04**	0.21*	0.52*	0.014**	0.319**

* P<0.06, ** P<0.001 Compared as Group I Vs Group II, Group I Vs Group III
Spg, Spermatogonia; R-Spc, Resting Spermatocyte; Pachy, Pachyten Spermatocyte; 7-Sptd, 7-Stage Spermatids

the testicular tissue was noted in Gr. III (Fig. 1c). Therefore, postmeiotic spermatogenic arrest and degeneration of spermatogenic cells in BHC fed rats at a dose of 6 mg/kg body weight over a period of 180 days indicated the toxic effects of BHC in testicular tissue. The significant amount of BHC residue in testis alongwith spermatogenic inhibition suggested that BHC was able to cross blood-testis barrier and led to testicular damage.

REFERENCES

1. Matsumura F. Differential toxicities of insecticides and halogenated aromatics. *Pergamon press*, 1984; pp 485-86.
2. Wester PW, Conten JH, Bisschop A. Histological study of *Poccilla reticulata* (Guppy) after long term ß-hexachlorocyclohexane exposure. *Aqua Toxicol* 1985; 6 : 271-76.
3. Philip GH, Reddy PM, Ramamurthi R. Changes in the protein metabolism in liver and kidney of *Mus booduga* gray after oral BHC feeding. *Bull Environ Contam Toxicol* 1988; 41 : 822-25.
4. Gautam AK, Gandhi DN, Jani JP, Bhatt HVK, Roy Chowdhury A. Histological and pharmacological changes in vas defferens of rats exposed to Hexachlorocyclohexane. *Res Comm Chem Pathol Pharmacol* 1989; 63 : 463-66.
5. Debruin A. Biochemical toxicology of environmental agents. *Elsevier/North Holand Biomedical Press Amsterdam* 1976; 310-12.
6. Gupta SK, Patel JS, Shah MP, Jani JP, Chatterjee SK, Kashyap SK, Organo-chlorine insecticide residues in fat of people from urban centres in India. *Pesticide*. 1980; 14 : 3-4.
7. Leblond CP, Clermont Y. Definition of the stages of the cycle of seminiferous epithelium in the rat. *Ann New York Acad Sci* 1953; 55 : 548-73.
8. Roy Chowdhury A, Rao RV, Gautam AK, Kashyap SK. Functional changes of testes in lead intoxicated rats. *Indust Hlth* 1987; 25 : 55-62.
9. Dikshith TSS, Datta KK. Effect of intra testicular injection of lindane and endrin in the testis of rats. *Acta Pharmacol Toxicol* 1972; 31 : 1-10.
10. Roy Chowdhury A, Bhatt HVK, Gautam AK. Testicular changes of rats under lindane treatment. *Bull Environ Contam Toxicol* 1987; 38 : 154-56.