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Reproductive Toxicity of Endosulfan in Male Albino Rats

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Pesticides have brought about the green revolution in the world and are being widely used to control agricultural pests and pests causing public health problems. Endosulfan (Thiodon 6,7,8, 9,10,10-hexachloro-1,5,5a,6,9,9a – hexa hydro – 6, 9 – methano – 2, 4, 3 – benzodioxathiepin – 3 oxide) a broad spectrum organochlorine pesticide is widely used in agriculture sector as a potent pesticide all over the world. Although, the US Environmental protection Agency (USEPA) has classified it as a highly hazardous pesticide (PANAP). Studies have been conducted on toxicity of endosulfan. (Reuber 1981, Ansari *et al.* 1984, Naqvi 1993, ATSDR,1991). However literature on reproductive toxicity in males, specifically in mammals is scarcely obtained. Therefore, this study is made to see the toxic influence of endosulfan on spermdynamics, testicular biochemistry and serum testosterone level of male rat.

MATERIALS AND METHODS

The Sprague Dawley albino rats (*Rattus norvegicus*) obtained from Hamdard University, New Delhi were housed in plastic cages at room temperature ($20^{o}\pm5^{o}C$) and uniform light (14:10:L:D). They were fed on standard laboratory Chow (Ashirwad Food Industries Ltd., Chandigarh, India) and fresh water *ad-libitum.* Technical endosulfan (α and β isomers in the ratio of 70:30) obtained from Hoechst, Bombay, India was used for experimentation.

Proven fertile healthy male rats (Weighing 150 - 250 gms) were divided into seven groups of five animals each. The control group I received only the vehicle (ground nut oil) whereas the animals of group–IIA,B, IIIA,B and IV A,B were administered orally endosulfan dissolved in ground nut oil by pearl point needle at the dose levels of 5,10 and 15 mg/Kg. b.wt./day for 15 and 30 days. The male rats were kept for fertility test on day 10-15 in 15 day's exposure and on day 25-30 in 30 days endosulfan exposure. The rats were cohabited with normal adult proestrus females in the ratio of 1:4. Successful mating was confirmed by presence of sperms in the vaginal smears. Females were separated and resultant pregnancies were noted when dams gave birth. The number and weight of litters were recorded. Fertility was calculated in control as well as in treated groups. The animals were weighed and autopsied under light ether anesthesia, sperm motility in cauda epididymis and density of testicular and cauda epididymis suspended sperm were calculated (Prasad *et. al.* 1972). The wet weight of the testes and other sex accessory organs was recorded after removing the adherent tissue and was frozen for measurement of glycogen

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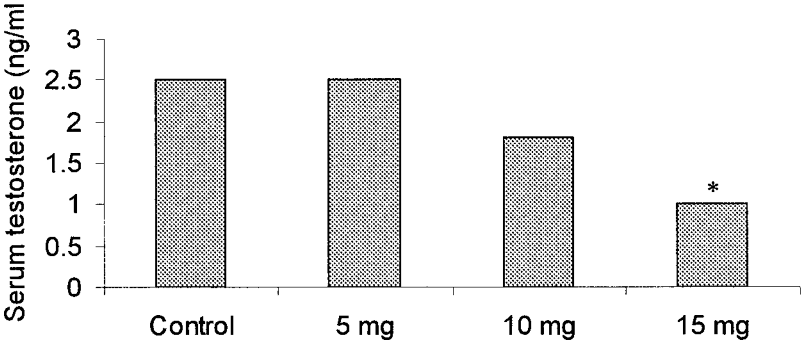

Figure 1. Endosulfan exposure in mg/kg bwt/day for 30 days. (* = P < 0.01)

(Montogomery 1957), Cholesterol (Zlatkis *et. al.* 1953), Protein (Lowry *et. al.* 1951) and Sialic acid (Warren 1959).

Serum was separated from blood by centrifugation at 3000 rpm and stored at $-20^{o}C$. Testosterone concentration was measured by radio immunoassay. (Belanger *et al* 1980). The data were analyzed statistically by one-way analysis of variance (ANOVA) and the significance of differences was set at $P<0.001$.

RESULTS AND DISCUSSION

The observations obtained after oral administration of endosulfan at various dose levels are shown in the table 1 and 2. A significant reduction in the weights of testes and sex accessory organs was observed. The spermatozoal motility in cauda epididymis and spermatozoal density in cauda epididymis and testes were significantly decreased in a dose dependent manner. A sharp decline in fertility (80% negative) in endosulfan treated rat was also observed at 15 mg dose level. The testicular biochemistry showed depletion of glycogen, sialic acid and elevation of testicular protein and cholesterol, whereas the reduction in the serum testosterone concentration were much pronounced at higher doses of endosulfan exposure (fig. 1)

The decreased sperm motility and density after oral administration of endosulfan at various dose levels is may be due to androgen insufficiency, (Singh and Pandey, 1989, Chitra *et al* 1999) which caused impairment in testicular functions by altering the activities of the enzymes responsible for spermatogenesis, this clearly suggests an antiandrogenic effect of endosulfan (Sinha *et. al.* 1995, Reuber, 1981). It is supported by the reduction in the serum testosterone at higher dose, which clearly demonstrated the inhibitory effects of endosulfan like other chlorinated insecticides (Wango *et al* 1997) on the secretion of pitutary gonadotrophins (FSH and LH) and in testes of rats (Singh and Pandey 1990).

Depletion of glycogen reserves in the testes attributes to the inhibition of glycogenolysis (Murty and Devi, 1982). Since glycogen is an energy source for general metabolism and constant supply of glucose is essential for proper functioning of testes. Similar decrease in the levels of Sialic acid also shows the necrotic condition of testes (Levinsky *et al* 1983). and the cause of reduction in sialic acid level is due to inhibition of spermatogenesis.

Table 1. Effect of endosulfan on sperm motility and sperm density of rat.

Treatment	Sperm motility (%) (Cauda)	Sperm Density (Million/ml^3) (Testes)	(Cauda)
Control IA	69.61 (±3.58)	4.15 (±0.06)	21.70 (±0.37)
Endosulfan 5 mg/Kg.b.wt./day IIA (15 days) IIB (30 days)	59.11 (±2.35) 45.21* (±2.7)	2.71* (±0.37) 0.87* (±0.13)	20.69 (±0.32) 12.14* (±1.80)
Endosulfan 10 mg/Kg.b.wt./day IIIA (15 days) IIIB (30 days)	51.08* (±3.11) 37.36* (±1.75)	0.86* (±0.11) 0.72* (±0.05)	14.15* (±0.39) 21.38 (±1.61)
Endosulfan 15 mg/Kg.b.wt./day IVA (15 days) IVB (30 days)	16.15* (±1.52) 16.04* (±2.75)	1.25* (±0.25) 0.87* (0.23)	7.02* (±1.27) 6.35* (±0.35)

Values given are Mean of results obtained from 5 animals.
Figures in parenthesis indicate ± SE of mean
* = Significant ($P \leq 0.001$)

Table 2. Biochemical changes in the testes of rat after oral administration of endosulfan.

Parameters	Control	5 mg/Kg.b.wt./day		10 mg/Kg.b.wt./day		15 mg/Kg.b.wt./day	
		IIA (15 days)	IIB (30 days)	IIIA (15 days)	IIIB (30 days)	IVA (15 days)	IVB (30 days)
Glycogen (mg/gm)	2.70 (±0.13)	1.00* (±0.22)	0.92* (±0.23)	0.46* (±0.06)	0.43* (±0.01)	1.05* (±0.06)	0.58* (±1.8)
Sialic Acid (mg/gm)	5.10 (±0.19)	4.10* (±0.22)	4.11 (±0.10)	4.29* (±0.10)	4.33* (±0.12)	4.10 (±19.57)	4.20 (±0.21)
Protein (mg/gm)	255.30 (±17.20)	327.96 (±23.02)	354.62* (±0.44)	313.30 (±21.03)	337.74* (±15.92)	349.98* (±15.22)	386.64* (±11.89)
Cholesterol (mg/gm)	5.92 (±0.41)	5.46 (±0.83)	6.26 (±0. 85)	7.48 (±1.19)	8.5* (±0.28)	9.75* (±0.47)	9.50* (±1.19)

Values given are Mean of results obtained from 5 animals.
Figures in parenthesis indicate ± SE of mean
* = Significant ($P \leq 0.001$)

The elevation in the testicular protein (Gupta *et al.* 1981, Singh and Pandey 1989) may be due to the hepatic detoxification activities caused by endosulfan which results in the inhibitory effect on the activities of enzyme involved in the androgen biotransformation, (Dikshith and Dutta 1972). Similar elevation in protein content caused by other organochlorine has also been reported. (Shivanandapp *et. al.* 1981, Bhatnagar and Malviya, 1986). The accumulation of cholesterol (Braze 1976) in the testes is a direct evidence of antiandrogenic action (Murugravel and Akbarsha, 1991). Since cholesterol being an important precursor in the synthesis of steroid hormones (Turner and Bagnara, 1978) its requirement for normal activities of the testes has been well established.

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