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Effect of Endosulfan on the Testis of Growing Rats

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Endosulfan, a chlorinated cyclodiene compound is used worldwide as insecticide & acaricide for the control of various pests in agriculture. The effect of endosulfan on laboratory animals reported so far is restricted to immunological, neurological and genotoxic studies (Toxicological Profile 1993). Few reports on the effect of endosulfan on reproductive functions are also available but are restricted to adult animals only (Dikshith et.al. 1984; Singh and Pandey, 1990; Sinha et.al., 1995). No report is available on the effect of endosulfan on the testicular maturation. This is significant in view of the fact that young growing animals are more susceptible to the toxicity of xenobiotics (Sjoberg et. al., 1985;Saxena et.al .,1990). The risk of exposure to any xenobiotic during the prepubertal stage of sexual maturity may lead to permanent damage to the gonads. Therefore, in the present study we have studied the effect of various doses of endosulfan on growing rats from weaning to 90 days of age to see the effect of this pesticide on the testis attaining sexual maturity.

MATERIALS AND METHODS

Twenty weaned male Druckrey rats (3 weeks old; 48-50g b.wt) were procured from the Industrial Toxicology Research Centre colony. The rats were divided into four groups of five rats each. Group II, III and IV were given endosulfan (95.32% purity) at a dose of 2.5, 5.0 or 10 mg/kg body weight in 0.2 ml peanut oil through oral incubation (Pandey et.al., 1990; Sinha et. al., 1995). Group I served as control and was given peanut oil only. The treatment was carried out till 90 days of age, the dose schedule being five days a week. Body weight was monitored twice a week. The animals were housed in stainless steel cages in an air conditioned room where regular alternate cycles of 12 h light and darkness were maintained and supplied with pelleted diet (Lipton India Ltd., animal feed, India) and tap

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water ad libitum. After the completion of treatment, the animals were sacrificed by cervical dislocation. Testes were dissected free, weighed and processed for enzymatic analysis and intratesticular spermatids count . The epididymis were dissected out, their caudal end teased in saline for sperm count and sperm abnormality. A portion of the testis was homogenized in 0.32 M sucrose for estimating lactic dehydrogenase (LDH) and sorbitol dehydrogenase (SDH); in 0.5 M Tris buffer for gamma glutamyl transpeptidase (GGT) and in 0.2 M Triethanolamine buffer for glucose-6-phosphate dehydrogenase (G6PDH). The homogenates were centrifuged at 12000 x g at 4°C for 20 min. to obtain a clear post-mitochondrial fraction. The activities of SDH, LDH, GGT and G6PDH were measured in the supernatant according to methods of Kornberg (1955), Gerlach (1983), Roomi and Goldberg (1981) and Lohr and Wailer (1974) respectively. The activities were recorded in a spectronic-2000 spectrophotometer with attached printer (Bausch and Lomb model). Protein in the samples were measured according to the method of Lowry et.al. (1951).

The sperm count and morphology were performed by dissecting out the cauda epididymis and teasing it in a known volume of normal saline at 37°C. Sperm counting was done using a haemocytometer according to the method of Feustan et.al (1989). For observing morphological changes in heads and tails to assess sperm abnormality, a drop of 1% Eosin Y was added to the sperm suspension and kept for 5 minutes at 37°C. After that a drop of sperm suspension was placed on a clean slide and spread gently to make a thin film. The film was air dried and then observed under a microscope for changes in sperm morphology according to the method of Feustan et.al (1989). The criteria chosen for head abnormality were - no hook, excessive hook, amorphous , pin and short head. For tail, the abnormalities recorded were - coiled flagellum, bent flagellum, bent flagellum tip. The result presented are the percentage overall.

For intratesticular spermatids count,testis was dissected free, weighed and the tunics albugenia was gently stripped from the parenchyma. The tissue was then homogenized in an aqueous solution containing 150 uM sodium azide and 0.05 % (v/v) Triton X-1OO. The homogenate was then divided into different aliquots of appropriate dilutions. A drop of homogenate was placed on a haemocytometer and observed under microscope for counting and calculating the number of homogenization/sonication resistant spermatid nuclei per ml testis suspension. For calculating daily sperm production, the values obtained were divided by 6.1 (Robb et al. 1978).

Data were analysed by one way Analysis of Variance (ANOVA) after ascertaining the homogeneity of variance and normality assumptions of the data (Brunning and Kintz 1977). Intergroup comparison was done by computing Least Significant Difference. The significance level was ascertained at $p<0.05$.

RESULTS AND DISCUSSION

No death were recorded in any of the treated group throughout experimentation. Neither the body nor the testis showed any change in weight as compared to the control group. Table 1 reveals the effect of endosulfan on sperm count and sperm morphology. A dose dependent reduction in the number of sperm count was recorded in all the group. The percent decrease observed were 39, 62 and 75 % for 2.5, 5.0 and 10.0 mg/kg endosulfan respectively as compared to the control group. The intergroup comparison also showed a significant decrease ($P(0.001$) with higher dosed groups being most toxic. A significant percent increase in the sperm morphology in all the treated group was observed. However, the changes were less ($P<0.05$) in lowest dosed group but increased with the increase in the dose level at 5.0 and 10.0 mg/kg of endosulfan ($P<0.00l$).

Marked depletion in the number of spermatids as well as decrease in daily sperm production was observed in all the treated group as compared to the control. The intercomparison of the treated group showed that the toxicity was in a dose dependent manner with the higher dose exhibiting maximum depletion compared to the preceding group and the control.

Table 2 shows the effect of endosulfan (0, 2.5, 5.0 or 10.0 mg/kg body weight) on the testicular marker enzymes . A significant elevation in the activities of the enzymes LDH, GGT and G6PDH was recorded in all the treated group in a dose dependent manner ($P<0.001$). The degree of elevation being maximum in the highest dose group i.e. 10 mg/kg body weight (95.73% for LDH, 50.19% for GGT and 45.43 % for G6PDH) as compared to the control. However, SDH registered a decrease in its activities in a dose ($p(O.001$) dependent manner, the highest group showing maximum effect (58.94 %) as compared to the controls.

Endosulfan has been reported to interfere in the spermatogenic (Sinha et. al. 1995) and steroidogenic cycle in adult rats (Singh and Pandey, 1989, '90). The present study was carried out to see the effect of endosulfan exposure during prepuberty on spermatogenesis in the testes of growing rats to see

its role in inducing damage to gonads attaining sexual maturity.

In the present study, activities of marker enzymes viz. LDH, SDH, GGT and G6PDH are considered to be functional indicators of spermatogenesis. LDH and SDH are associated with the maturation of germinal epithelial layer of seminiferous tubules and associated with post meiotic spermatogenic cells. LDH is widely distributed and located in the tubules and appears with pachytene spermatocyte formation (Shen and Lee 1976, Blackshaw, 1970). A high concentration of LDH is present in the testes of newborn rats and its activity declines with the development of testis (Free, 1970). similarly, SDH is associated with pachytene stage spermatocyte which increases rapidly with the maturation of the testes (Hodgen and Sherins, 1973). An increase in the activity of LDH and decrease in SDH after endosulfan exposure suggest an altered cellular physiology of the germinal elements in seminiferous tubules.

Gamma glutamyl transpeptidase is associated with Sertoli cell function and has been shown to exhibit an inverse relationship with spermatogenesis (Males and Turkington, 1971). An increase in the activity of GGT in the present study may be related to the degeneration of seminiferous tubules. Glucose-6-phosphate dehydrogenase is the marker of functional status of leydig cells - a site of steroid biosynthesis (Tamaoki et. al. 1975). G6PDH is regarded as the potential generator of NADPH which is required for hydroxylation in steroid biosynthesis. The activity of G6PDH changes from high to low levels as spermatogenesis proceeds (Shen and Lee, 1976). A significant increase in the activity of G6PDH after endosulfan exposure suggests that steroidogenic activity is being affected in the testes. Such changes have earlier been shown at the hormonal level by Singh and Pandey (1989) where they have reported an alteration in luteinizing hormone (LH) which is responsible for the production of male hormone testosterone. They have also observed changes in the pattern of the steroidogenic enzymes 3βHSD and 17 βHSD leading to inhibition of testicular androgen biosynthesis in adult rats fed with endosulfan at a dose of 5.0 and 10.0 mg/kg body weight after 15 and 30 days of exposure. The alteration in enzymes activity which has led to the destruction of seminiferous epithelium and loss of germinal elements has resulted in the reduction of the number of spermatids associated with the decrease in the daily sperm production in the testes. The low sperm production has further led to a decreased sperm release in the epididymis of the endosulfan treated rats as well as significant sperm abnormality in all the treated groups. When these

Table 1. Effect of endosulfan exposure on body and testis weight , sperm count, sperm abnormality, intratesticular spermatids count and daily sperm production in rats

	Control	ENDOSULFAN (mg/kg body weight) 2.5	5.0	10
Body weight (g)	225±5.65	230±10.5	238±8.5	225±6.35
Testis weight (g)	2.49±0.19	2.58±0.25	2.62±0.20	2.55±0.16
Sperm count (x 10^6/ml) cauda epididymis	58.08±2.30	34.96±0.54^{a}**	22.00 .28^{ab}***	14.12±0.52^{abc}***
Sperm abnormality (%)	6.29±0.07	6.74±0.07^{a}*	7.22±0.12^{a}***^{b}*	8.14±0.16^{abc}***
Spermatids count (x 10^6/ml testis)	105.60±1.94	82.80±2.42$^{=}$***	73.6±l.60^{a}***^{b}*	60.40±l.16^{abc}***
Daily sperm production (x 10^6/ml testis homogenate)	17.32±0.32	13.57±0.40^{a}***	12.13±0.23^{a}***^{b}**	9.89±0.19^{abc}***

Values represent mean ± SE of 5 animals/group.

The significance of the difference among different groups was evaluated by applying one-way ANOVA followed by Student's 't' test (Brunning and Kintz, 1977).

a = versus control; b = versus 2.5 mg; c = versus 5.0 mg

*p<0.05; **<0.01 and ***<0.001

Table 2. Effect of endosulfan on rat testicular enzymes.

Dose (mg/kg body wt.)	LDH (nmol NADH oxidised/ (min/mg protein)	SDH (nmol NADH oxidised/ min/mg protein)	GGT (nmol p-nitroaniline liberated/min/mg protein)	G6PDH (nmol NADP reduced/ min/mg protein)
Control	356.40±9.75	10.82±0.14	10.65±0.11	10.96±0.11
2.5	479.60±13.10^{a}***	8.55±0.13^{a}***	13.22±0.13^{a}***	12.54±0.19^{a}***
5.0	588.40±9.54^{ab}***	6.87±0.10^{ab}***	14.34±0.20^{ab}***	13.90±0.10^{ab}***
10.0	697.60±13.13^{abc}***	4.88±0.10^{abc}***	15.99±0.12^{abc}***	15.94±0.10^{abc}***

Values represent mean ± SE of 5 animals/group.

The significance of the difference among different groups was evaluated by applying one-way ANOVA followed by Student's 't' test (Brunning and Kintz, 1977).

a = versus control; b = versus 2.5 mg; c = versus 5.0 mg

***p<0.001

LDH = Lactic dehydrogenase; SDH = Sorbitol dehydrogenase; GGT = Gamma glutamyl transpeptidase and G6PDH = Glucose-6-phosphate dehydrogenase.

results were compared to our earlier work (Sinha et. al. 1995) where the effect of endosulfan on testes of adult male rats were studied it was observed that the exposure to this pesticide during adult life had its effect at a dose of 5 and 10 mg/kg body weight and was not in a dose dependent manner. Whereas endosulfan when administered during prepubertal age has its damaging effect from the lowest dose (2.5 mg) and the toxicity increased with the increase of the dose level.

Hence, it can be concluded that endosulfan exposure during growing age i.e. during the period of testicular maturation when spermatogenesis is under progress may result in disturbed spermatogenesis at sexual maturity.

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