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Dicofol Formulation Induced Toxicity on Testes and Accessory Reproductive Organs in Albino Rats

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Dicofol (Kelthane) 2,2, 2-trichloro-1, 1-bis (4-chlorophenyl ethonol) is used extensively on nuts, apples, citrus fruits, tea and cotton crops in India. It is structurally similar to DDT. There are little published work on the specific toxic effects of dicofol in experimental animals. Some small adverse effects associated with reproduction in rats and mice have been reported (Brown 1972). A detailed study of the protection of workers in Florida citrus groves from contamination by dicofol has been reported (Nigg et al. 1986). Sobti et al. (1983) reported that dicofol has cytokinetic and cytogenetic effects on human lymphoid cells in vitro. Much data have been reported on the reproductive toxicity of chlorinated hydrocarbons. (Burlington and Linderman 1950; Singh and Pandey 1989). Shivanandappa and Krishnakumari (1983) have reported that the treatment with hexachlorocyclohexane induced testicular dysfunction with marked testicular atrophy, reduced testicular size and spermatogenic arrest in rats. It has been stated that inhibition of spermatogenesis and fatty degenerative changes in Sertoli cells were major findings in male adult rats exposed to methoxychlor (Ball, 1984). Keeping these points in view, the present investigation was undertaken to study the effect of dicofol on testes and accessory reproductive organs in albino rats.

MATERIALS AND METHODS

The sample of dicofol (Kelthane) used in this study was commercial chemical supplied by United Phosphorus Ltd., Mumbai obtained from the local company's market containing 18.5% (W/W) [minimum purity 67.12%, emulsifier (Noigen DF 0102, 9% ww) Xylene (dimethyl benzene 36% ww) aromex 36.5% ww)] and dissolved in olive oil. Doses were given below the acute LD_{50} level of intoxication.

Normal male albino rats of Wistar Strain, aged 90-120 d old and weighing between 200-220 gms were used for the experiments. The rats were maintained in the normal day/night (12/12hr) schedule at room temperature 26±1°C. Daily body weight recorded in the morning before the administration of dicofol. The stock solution was prepared in olive oil to the required concentrations for oral administration. The rats were divided into 5 groups, each consisting of 6 animals. Graded doses of dicofol from 200, 300, 400 and 500 mg/kg B.wt/d was administered orally for 30 d. Olive oil treated normal rats served as controls. All

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the animals were killed by cervical dislocation and necropsied on 31st d, 24 hr after the last dose. The testes, epididymides, vasa deferentia, seminal vesicles, prostate gland, Cowper's glands, coagulatory glands were dissected out, freed from the adherent tissues and weighed to the nearest milligram. Liver, kidneys, adrenals, thyroid and thymus were also dissected out and weighed. The right testis and epididymis were fixed in Bouin's fluid, embedded in paraffin, sectioned at 6μm thickness and stained with hematoxylin and eosin (Humason 1979). From each testis, 20 sections were randomly selected from each group. Histometric observations were made with an occulometer. The histometric data are the number of seminiferous tubules in microscopic field, diameter of seminiferous tubules, diameter of spermatogonia, spermatocytes, spermatids and Leydig Cells, height of the Sertoli cells, number of spermatogonia, spermatocytes and spermatids. Data were subjected to analysis of variance (ANOVA) together with Dunnett's test by comparing data from the control group with those of the dicofol treated groups ($P<0.05$).

RESULTS AND DISCUSSION

There was no significant change in the weight of the testes with 200, 300 and 400 mg/kg/d dicofol treatment. But there was a significant decrease in the weight of the testes with 500 mg/kg/d dicofol treatment when compared with that of the control. Treatment with 200 and 300 mg/kg/d dicofol showed no significant change in the diameter of seminiferous tubules, spermatogenic cells, Leydig cells and height of the Sertoli cells. However, there was a significant decrease in the diameter of seminiferous tubules, spermatocytes, Leydig cells with 400 mg/kg/d dicofol treatment. There was a significant reduction in the diameter of seminiferous tubules, spermatogonia, spermatocytes, Leydig cells and height of the Sertoli cells with 500 mg/kg/d dicofol treatment when compared with that of the control rats (Table 1).

The number of spermatogonia, spermatocytes, spermatids and Leydig cells were decreased significantly with 400 and 500 mg/kg/d dicofol treatment. However, there was no significant change in the number of seminiferous tubules, spermatogenic cells and Leydig cells with 200 and 300 mg/kg/d dicofol treatment (Table 2). There was no significant change in the weight of vasa deferentia, seminal vesicles, prostate glands with 200, 300 and 400 mg/kg/d treatment. But there was a significant decrease in the weight of epididymides when compared with that of the control rats. There was no significant change in the body and non-reproductive organs weight in all the dicofol treated rats. Histologic observation of the testis of the control rats reveals normal spermatogenesis (Figure 1A). Histologic structure of the testis of the rats treated with 200 and 300 mg/kg/d dicofol shows apparently normal spermatogenesis (Figures 1B and C). Histologic examination of the testis of the rats treated with 400 and 500 mg/kg/d dicofol shows arrest of spermatogenic stages (Figures 1D and E). Histologic observations of epididymis shows normal structures in control and 200, 300 and 400 mg/kg/d dicofol treated rats. However, treatment with 500 mg/kg/d dicofol shows loose arrangement of tubules with intertubular spaces. The sperms are concentrated at the centre of the lumen and are less in number.

The testes of humans and other mammals are highly susceptible to damage produced by genetic disorders, environmental or occupational exposure to chemicals or other means. Specific causes of testicular damage have been catalogued (Jackson and Ericsson 1970; Jackson 1973; Gomes 1977). Quantity and quality of sperm production has been adversely affected following exposures of certain drugs and chemicals, particularly mutages and teratogens. There is a clear correlation between the degree and duration of exposure to pesticides and the extent of spermatogenic arrest and hormonal imbalance. Testicular atrophy and degenerative changes in the seminiferous tubules have been reported in experimental animals administered with various organchlorine and organophosporous insecticides (Dutta and Dikshith 1973; Dikshith et al. 1978; Nigam et al. 1979).

The present findings revealed that there was a significant decrease in the weight of the testes and epididymides with higher doses of dicofol treatment. Histometric data obtained in the present study has also revealed a significant decrease in the diameter of seminiferous tubules, spermatogonia, spermatocytes, Leydig cells and height of the Sertoli cells with higher doses of dicofol treatment. The number of spermatogonia, spermatocytes, spermatids and Leydig cells were also decreased significantly with higher doses of dicofol treatment. Histologic structure of the testis revealed that there is an arrest of spermatogenic stages with higher doses of dicofol treatment. Histologic observations of the epididymis showed no compact arrangment of the tubules and intertubular spaces. The sperm are concentrated more at the center of the lumen and were decreased in number with higher doses of dicofol treatment. However, there was no significant change in the weight of the testes, epididymides, and diameter of seminiferous tubules, spermatogenic cells, Leydig cells and height of Sertoli cells with low doses of dicofol treatment. Histologic structure of the testis showed normal spermatogenesis with lower doses of dicofol treatment. Histologic observations of the epididymis also showed normal structures as in control rats. There was no significant change in the weight of vasa deferentia, seminal vesicles, prostate gland, coagulatory glands, Cowper's glands and ampullary glands and also the body weight and non-reproductive organs weight in all the doses of dicofol treatment.

Similar, results were obtained in other chlorinated hydrocarbon pesticides, which are reported to inhibit testicular growth, arrest of spermatogenic activity and decrease in the epididymis weight. Changes in the testes involve the tubules and not the interstitial tissues, and they have been attributed to an estrogen – like action of DDT. It has been suggested that inhibition of spermatogenesis and fatty degenerative changes in Sertoli cells and in spermatogonia and spermatocytes were major findings in male adult rats exposed to methoxychlor (Ball 1984). It has been stated that lindane and technical BHC have effects on the testes of rats and mice (Van velsen et al. 1986). Shivanandappa and Krishnakumari (1983) have demonstrated that the treatment with hexachlorocyclohexane induced testicular dysfunction in rats with marked testicular atrophy reduced tubular size, spermatogenic arrest. It has been reported that the weight of testes was reduced at high dose level of endosulfan, the weight of the epididymides, coagulatory glands, seminal vesicles and ventral prostates were decreased at all dose levels and the

Table 1. Effect of dicofol on weight of the testes, diameter of seminiferous tubules, spermatogenic cells, Leydig cells and height of the sertoli cells in albino rats

Groups	Treatment (mg/kg/day)	Number of rats	Testes wt. (g/100g B.wt)	Seminiferous tubules, Spermatogenic cells and Leydig cells size μm (diameter) : M± S.E.					Height of sertoli cells (μm)
				Seminiferous tubules	Spermatogonia	Spermatocytes	Spermatids	Leydig cells	
A	Control -oil	6	1.3 ± 0.0	395.0 ± 11.2	11.2 ± 1.2	8.2 ± 0.5	7.8 ± 2	9.1 ± 0.4	8.6 ± 1.2
B	200	6	1.4 ± 0.1	378.5 ± 3.5	10.0 ± 0.2	7.5 ± 0.0	6.6 ± 4.1	8.2 ± 4.1	6.4 ± 0.2
C	300	6	1.2 ± 0.5	364.9 ± 8.4	9.9 ± 0.1	7.8 ± 0.0	6.7 ± 2.1	6.4 ± 2.2	6.3 ± 0.0
D	400	6	1.1 ± 0.1	331.5 ± 3.3*	9.5 ± 0.1	6.6 ± 0.1*	8.0 ± 1.0	6.6 ± 0.3*	6.1 ± 0.2
E	500	6	1.0 ± 0.1*	325.7 ± 6.4*	7.9 ± 0.1*	5.5 ± 0.0*	5.0 ± 2.0	5.5 ± 0.3*	4.5± 0.0*

M ± S.E. = Arithmetic mean ± standard error
*= significant P<0.05 compared to control

Table 2. Effect of dicofol on the number of seminiferous tubules, spermatogenic cells, Leydig cells and weight of the epididymides in albino rats

Groups	Treatment (mg/kg/day)	Number of rats	Number of semeniferous tubules in microscopic field	Number of spermatogenic cells; M ± S.E					Epididymides wt. (g/100g B.wt)
				Spermatogonia	Spermatocytes	Spermatids	Sertoli cells	Leydig cells	
A	Control - oil	6	23.4 ± 2.6	121.5 ± 2.4	701.5± 1.4	1010.4 ± 7.1	21.1 ± 0.4	44.2 ± 1.1	0.347± 0.03
B	200	6	21.6 ± 2.0	121.4 ± 3.8	700.0 ± 1.2	1002.0 ± 6.0	20.0 ± 0.5	40.0 ± 2.2	0.373± 0.02
C	300	6	21.6 ± 1.6	120.6 ± 8.4	690.0 ± 5.7	1000.0 ± 7.0	20.5 ± 0.3	40.0 ± 2.3	0.319± 0.03
D	400	6	20.0 ± 0.4	112.7 ± 2.0*	683.0 ± 1.6*	992.0 ± 2.4*	19.5 ± 0.6	37.2 ± 0.2*	0.294± 0.01
E	500	6	19.2 ± 0.2	98.8 ± 2.6*	680.1 ± 2.4*	975.0 ± 3.2*	20.0 ± 0.5	38.0 ± 0.0*	0.249± 0.009*

M ± S.E. = Arithmetic mean ± standard error
*= significant $P<0.05$ compared to control

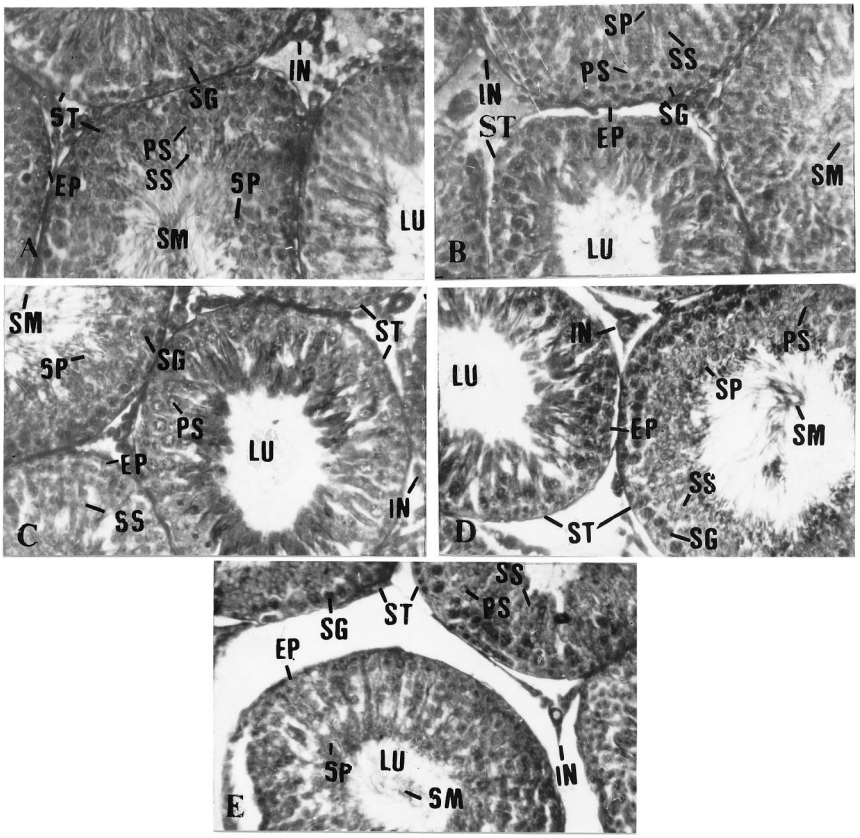

Figure 1: A through E. Effect of dicofol on testes in albino rats

A - T S of the testis of the control rat showing normal structure of seminiferous tubules and spermatogenic cells.

B - T S of the testis of the rat treated with 200 mg/kg/d dicofol for d 30, showing normal structure of seminiferous tubules and spermatogenic cells.

C - T S of the testis of the rat treated with 300 mg/kg/d dicofol for d 30, showing normal structure of seminiferous tubules and spermatogenic cells.

D - T S of the testis of the rat treated with 400 mg/kg/d dicofol for d 30, the number of spermatogenic cells are reduced.

E - T S of the testis of the rat treated with 500 mg/kg/d dicofol for d 30, showing the decrease in the number of spermatogenic cells.

Abbreviations: ST = Seminiferous tubule; EP = Epithelium; LU = Lumen; IN = Intertubular tissue; SG = Spermatogonia; PS = Primary spermatocytes; SS = Secondary spermatocytes; SP = Spermatids; SM = Sperms. [Harri's Haematoxylin and eosin; X 400].

biochemical changes were reversed when the endosulfan treatment was withdrawn (Gupta and Chandar 1977; Singh and Pandey 1990). The present investigation is comparable to other chlorinated pesticides on account of exhibiting estrogenic activity of dicofol which leads to decrease in the weight of the testes, epididymides and arrest of spermatogenesis (Burlington and Linderman 1950; Harris et al. 1974; Linder et al. 1983; Shivanandappa and Krishnakumari 1983; Ball 1984; Singh and Pandey 1990;). The present study on the dose effect of dicofol on the histologic structure of testis also revealed two principal impacts on the male reproductive system of albino rats namely, the antispermatogenic and antiandrogenic effects. The antispermatogenic adverse effect is reflected in the arrest of spermatogenesis as seen on the diameter, total count of spermatogonia, spermatocytes, spermatids and height of the Sertoli cells and cell debris in the lumen of the semniferous tubules. The antiandrogenic action of dicofol reflected in the regression of Leydig cells diameter and number and also the reduction in the height of Sertoli cells. The adverse effects on the structure of epididymal epithelium and weight of the epididymides in our study possibly suggest the anitandrogenic property of the dicofol. It gives a clue that high doses of dicofol treatment affect the spermatogenesis showing antispermatogenic and antiandrogenic property directly or indirectly. The above results suggest that dicofol mimics estrogenic activity when compared to other chlorinated pesticides may have a direct effect on the testes or indirectly through the hypothalamo-hypophysial-testicular axis. Whether the observed toxicity occured as a result of direct effects upon the testes or whether indirectly through action on the hypothalamus and / or pituitary, or by desensitizing the testes to gonadotropins cannot be ascertained from this study. Further investigation on the mechanism of action of dicofol on testes toxicity will be necessary.

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