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Toxic Effects of Phosphamidon on the Testes of Swiss Albino Mice

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Phosphamidon, chemically known as phosphoric acid, 2-chloro-3-(diethylamino)-1-methyl-3-oxo-1-propenyl dimethyl ester, is an organophosphate, systemic insecticide-acaricide. It is extensively used against sucking, chewing and mining insects. Phosphamidon is strongly recommended for application in perennial crops and annual field plantation in India. Few attempts have been made to study the effects of organophosphorous insecticides such as ethyl parathion, methyl parathion, DDVP and diazinon on the reproductive organs of mammals (Dikshith and Datta, 1972; Datta and Dikshith, 1973; Krause and Homolo, 1974; Chakrawarty et al., 1981). No information seems to be available on the effects of phosphamidon on the mammalian testes. Since reports on its effects on carbohydrate metabolism, hepatotoxicity, teratogenecity, mutagenecity and carcinogenecity are now available (Epstein & Legator, 1971; Usha Rani et al., 1980; Bhatnagar and Soni, 1985; Omkar et al., 1986; Bhatnagar and Jain, 1986). The present study was, therefore, planned to evaluate its toxicity on the testes of albino mice after subchronic exposure.

MATERIAL AND METHODS

Swiss albino mice obtained from CDRI, Lucknow, were bred and housed in air-cooled room with natural daylight for 12-14 hours. The colony was maintained on standard mice feed procured from Hindustan Lever Limited, New Delhi. Tap water was provided ad libitum. Mature male mice were selected from the colony and divided into three groups - two experimental and one control. The animals of the two experimental groups were subjected to 35 ppm phosphamidon in drinking water for 30 and 60 days respectively. The control animals received plain tap water. After treatment, the animals of the respective groups were sacrificed and their testes removed and fixed appropriately for histopathological examination and biochemical estimation of glycogen (Montgomery, 1957), cholesterol (Zlatkis et al., 1953) acid and alkaline phospha-

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Table 1 : Biochemical observations in testis after sub-chronic treatment with Phosphamidon (35ppm)

	Glycogen (mg/g)	Cholesterol (mg/g)	Acid Phosphatase (mg pi/g/hr)	Alkaline Phosphatase (mg pi/g/hr)
CONTROL	0.37±0.03	28.15±0.76	2.59±0.26	5.21±0.37
30 DAYS	1.51±0.07**	21.13±0.21**	5.37±0.08**	3.56±0.36*
60 DAYS	0.48±0.05	21.66±0.49**	4.36±0.36*	4.74±0.32

* - Difference highly significant from the concurrent control value, $p < 0.05$

** - Difference highly significant from the concurrent control value, $p < 0.01$

tase (Fiske and Subbarow, 1925). The cauda epididymides and testes of the animals were freshly utilized to estimate the sperm density and percent motility of cauda epididymal spermatozoa (Prasad et al., 1972). The obtained data was statistically analyzed using Student's 't' test.

RESULTS

Biochemically, the investigated parameters exhibited more significant changes in the group of animals that was intoxicated for 30 days (Table 1). The glycogen content of testis after 30 days treatment with phosphamidon was 1.51 ± 0.07, which was very significantly higher than that of control (0.37 ± 0.03). This value however, dropped to almost normal, after 60 days. The activities of acid and alkaline phosphatases also exhibited more significant changes after shorter treatment than that for longer period i.e. 60 days. The cholesterol level in the tissue was however, reduced at both treatment intervals i.e. 21.13 ± 0.21 at 30 days and 21.66 ± 0.49 at 60 days as compared to the control value 28.15 ± 0.76.

The sperm density in the cauda epididymis remained unaltered by phosphamidon treatment in the present study (Table 2). The density in testis however was reduced significantly at 30 days (2.60 ± 0.12) whereas 60 days treatment exhibited a non-significant decrease in sperm density (2.81 ± 0.13) as compared to that of control (2.93 ± 0.16). The pesticide treatment produced no change in the motility of the cauda epididymal spermatozoa (Table 2).

Table 2. Effect of sub-chronic treatment of phosphamidon on sperm motility and sperm density.

	Control	30 days treatment	60 days treatment
Sperm motility (Cauda epididymis)	73.0±4.24	72.5±2.50	72.25±2.14
Sperm density million/ml (Cauda epididymis)	40.68±9.52	39.21±0.39	40.26±0.83
Sperm density millions/ml (Testis)	2.93±0.16	2.60±0.12*	2.81±0.13

*Difference significant from the concurrent control value, $P < 0.05$

Histologically, the testes of animals treated for 30 days exhibited pronounced cytoplasmic vacuolization and pycnotic nuclei in many germ cells. Few seminiferous tubules showed giant cells that appear to be derived from spermatocytes or

spermatids. Towards the central region of the testis, some tubules exhibited desquamated spermatogenic cells, residual sperms and residual bodies in their lumen (Figs. 1 & 2).

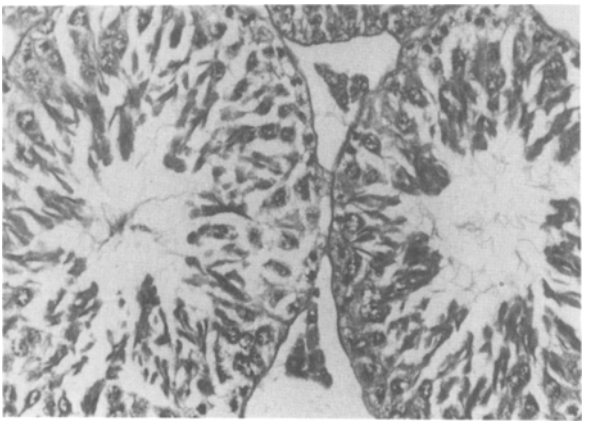

Fig. 1. Section of testis exhibiting vacuolization, residual sperms and residual bodies after 30 days treatment with 35 ppm phosphamidon. X 200.

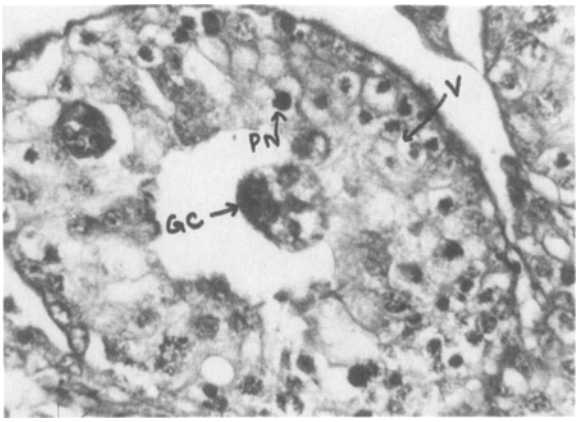

Fig. 2. Section showing giant cell (GC), pycnotic nuclei (PN) and cytoplasmic vacuolization (V). After 30 days treatment. X 200.

Treatment of mice for longer duration i.e. 60 days did not cause significant pathological changes in the testis. The germ cells at different stages of spermatogenesis appeared to be normal. Only in some peripheral seminiferous tubules, exfoliated cells were visible in the lumen (Fig. 3).

DISCUSSION

The toxic effects of phosphamidon seem to be more pronounced in male mice after their exposure for 30 days as compared to

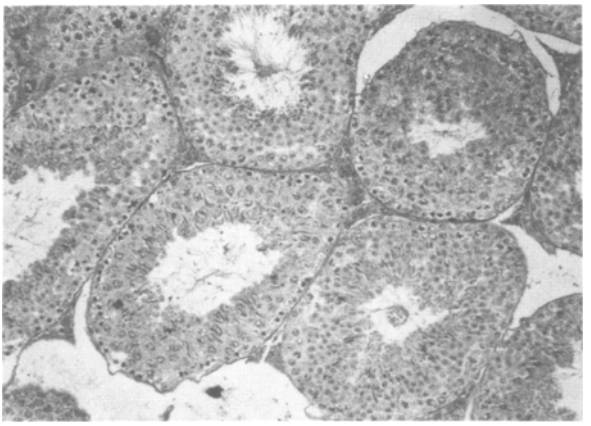

Fig. 3. Photomicrograph showing normal germ cells at various developmental stages. After 60 days treatment. X 50.

60 days treatment.

Histologically, certain degenerative changes were visible in the testis of mice exposed for 30 days. Corresponding to it, there occurred a decrease in the testicular sperm density (Table 2). The pesticide treatment, however, did not cause total depletion or immotility of sperms in the testis. It is also known that the sperms are stored in cauda epididymis after being produced in the testis. This may probably account for the unaltered density of the cauda epididymal spermatozoa in both the experimental groups.

The animals that were exposed to the pesticide for 60 days did not exhibit significant qualitative and quantitative changes in the testes. It thus appears that the animals developed some resistance when treated for longer period.

The toxicity of phosphamidon is also indicated by altered levels of glycogen, cholesterol and acid and alkaline phosphatase in the testis. Sub-chronic treatment of mice with phosphamidon for 30 days significantly elevated the glycogen level in testis. Nicander (1957) has demonstrated that its presence in the Sertoli cells and germinal tissue indicates that it serves as an energy substrate for the developing spermatids.

Histologically, 30 days treatment caused damage to the spermatogenic cells (Fig. 1) and quantitatively, the sperm density in testis was significantly low as compared to control (Table 2). This may account for less glycogen consumption by the spermatogenic cells and hence elevate its level in the testis. Prolonged treatment with 35 ppm phosphamidon produced no qualitative (Fig. 3) or quantitative changes (Table 2) in the tissue, thereby resulting in almost normal glycogen consumption in the testis after 60 days treatment and hence its normal value (Table 1).

Cholesterol functions as a precursor molecule during the synthesis of steroid hormones (Preidkalns and Weber, 1968).

Phosphamidon decreased the cholesterol level in the testis. This may cause reduced steroidogenesis. Eik-Nes and Hall (1962) and Dorfman et al. (1963) have earlier suggested that reduced androgen biosynthesis may be intimately related to the sperm output and fertility. This is also indicated by the decrease in the sperm density in testis. The lowered cholesterol level after 60 days treatment remains unexplained. Further exploration involving hormonal assay could probably account for this change.

Alkaline phosphatase is known to be responsible for the transference of metabolites across the plasma membrane (Elizabeth and Connell, 1972). It is also associated with the tissue growth, differentiation and secretory activity (Malone, 1960). A significant decrease in the activity of alkaline phosphatase occurred after 30 days treatment with phosphamidon. This decrease may be indicative of activities of enzymicidal and spermicidal nature in the tissue. Dikshith et al. (1975) have also reported decreased enzyme activity in rat testis with diazinon. On the other hand the level of acid phosphatase was elevated in the two experimental groups. This enzyme is known to be associated with the lysosomes, which participate in the intra- and extra cellular digestive processes (Novikoff, 1961). Riar et al. (1973) have also suggested that it is associated with scavenger activity. The increase in the enzyme activity may therefore be attributed to the lysosomal activity which in turn is associated with the degenerative changes in the germ cells of testis. Similar changes have also been reported with thimet and malathion in the testis of gerbil and rat (Saxena and Sarin, 1978; Chakrawarty et al., 1981).

It thus appears that phosphamidon exerts some structural and functional toxicity on the male gonads of mice when administered sub-chronically for a period of 30 days at the dose level of 35 ppm. But treatment for a longer duration i.e. 60 days leads to the development of resistance to the pesticide. Since phosphamidon did not cause severe toxicity to all the exposed animals, therefore continuous exposure to it for longer periods may be relatively less harmful than that for shorter periods.

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