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2,4-dichlorophenoxy acetic acid (2,4-d) induced histopathological changes in testes of a fresh water catfish, Heteropneustes fossilis (bloch)

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Abstract: Testis of *Heteropneustes fossilis* exposed to sub-lethal concentration of 2,4-D showed a number of degenerative changes. Marked histological changes were encountered in the testes of H.fossilis following herbicide poisoning. The testicular lobules exhibited degenerated spermatogonia and spermatocytes. The degenerative changes could either be on account of a direct toxic action of the environmental poison or due to an indirect effect via the hypothalamic pituitary-testicular axis. The interstitial cells of herbicide 2,4-D treated H.fossilis exhibited cytolysis, pyknosis and necrosis pointing to their reduced activity and consequent alteration of spermatogenesis. pointing to their reduced activity and consequent alteration of spermatogenesis. Active spermatogensis and formation of sperm were observed in testes from control fish whereas in the treated group only secondary spermatogonia and spermatocytes filled the tubular lumen. Necrotic areas were also evident in these testis. The interstitial cells of the control fish showed activation whereas those of the experimental were exhibiting signs of inactivity. Extensive necrosis in the spermatogenic and interstitial cells are known to be indicative of impaired gonadotrophic activity. Breaking and dissolution of the lobular wall in the testis of *H.fossilis* indicates a degenerative impact of the 2,4-D on this tissue.

Keywords: 2,4-dichlorophenoxy acetic acid, *Heteropneustes fossilis,* spermatogonia, spermatocytes, gonadotrophic activity

Introduction

Heteropneustes fossilis (Bloch.) is live catfish and inhabits all kinds of streams, irrigational channels marshes, swamps and even sewage-fed tanks. They always live in shoals showing wriggling and serpentine movements at the bottom under big boulders and among marshy beds. Occasionally they come close to the surface forming congregations particularly in the ponds or puddles covered with floating weeds or algal blooms, for gulping atmospheric oxygen. The frequency of the visits to the surface varies at different times of the day. It is very much dreaded for its poisonous pectoral spine and a poisonous gland at the base of spine. They are predacious and carnivorous. They belong to the family Heteropneustidae and order Siluriformes. 2,4-Dichlorophenoxy acetic acid (2,4-D) functions as a systemic herbicide and is used to control many types of broadleaf weeds. This compound is used in cultivated agriculture and in pasture and rangeland applications, forest management, home and garden situations and for the control of aquatic vegetation. It is the most widely used herbicide in the world. Widespread use of various pesticides, including 2,4-D and their consequent leaching to water, causes the aquatic ecosystem to get polluted and the populations of organisms, including fishes, suffer on account of the toxic nature of Herbicides. The ill-effect of 2,4-D not only harms the fishes but also all fish eating animals including human beings. Therefore, to avoid the pollution of aquatic ecosystems by 2,4-D, a sincere attempts is required for making their use judicious. Regular monitoring to maintain the level of pesticides within safe limits is evidently the most important (Basak and Konar, 1977a, 1977b). The present investigation is an attempt to evaluate the toxicological effects of 2,4-D on testes of *Heteropnuestes fossilis.*

Materials and Methods

Live specimens of the fish *Heteropneustes fossilis* (length 18.0+1 c m, weight 50.0 + 5 gm) were collected from local ponds at Singaramau, Jaunpur (U.P.) during the month September to February and were acclimatized with laboratory conditions for 15 days before experiments were set. The fishes were kept at controlled room temperature (24 + 1°C) in plastic aquaria containing continually aerated de-chlorinated tap water with dissolved oxygen contents 6.0 mg/L + 1.0 mg/L, pH 7.5 + 0.3 and hardness 160 mg/L (as $CaCo_3$). The fish were fed with minced goat liver on alternate days. Feeding was stopped 24 hour before the experiments began, and the fish were not fed during the experiments

Herbicide toxicity to fish: 24hrs-renewal bioassay test following APHA, AWWA, WPCF (1980) was performed to determine LC_{50} – 96 hrs values of 2,4-D using Spearman Karber method (Hamilton et.al., 1977) and was found to be 6 mg/liter. A group of 10 fishes were kept in different aquaria, each containing 10 litres of de-chlorinated tap water/ test solutions during the experiment. Fresh test solutions having desired concentration of the 2,4-D was made one hour prior to their renewal. During renewal of test solutions in the aquaria, due care was taken to keep the fishes undisturbed and to avoid handling stress. To remove test solutions from the aquaria siphoning method was applied. When tap water/test solutions reached to a minimum quantity, sufficient to keep the fishes undisturbed, the aquaria were simultaneously filled by gradual addition of fresh test solution. The process of siphoning was cut, and the simultaneous addition of fresh water/test solution, to the aquaria was continued for some period to ensure their complete replacement. The mortality of fishes, to calculate the LC_{50} and reaction of fishes to the 2,4-D at different intervals were regularly recorded. The fishes were considered dead, if these did not react to gently prodding and showed lack of respiratory movements. Dead fishes, if any, detected during the experiment, were immediately removed from the aquaria.

Experimental Design: Fishes are exposed to sublethal concentration (0.6 mg/litre) of 2,4-D. Fishes were cold anaesthetized following Mittal and Whitear (1978) and testes of fishes were excised ,rinsed in saline water and were fixed in 10% neutral formalin and Bouin's aqueous at 15 days, 30 days, 45 days, 60 days and 90 days of 2,4-D treatment. Standard methods of dehydration, clearing and embedding were used.

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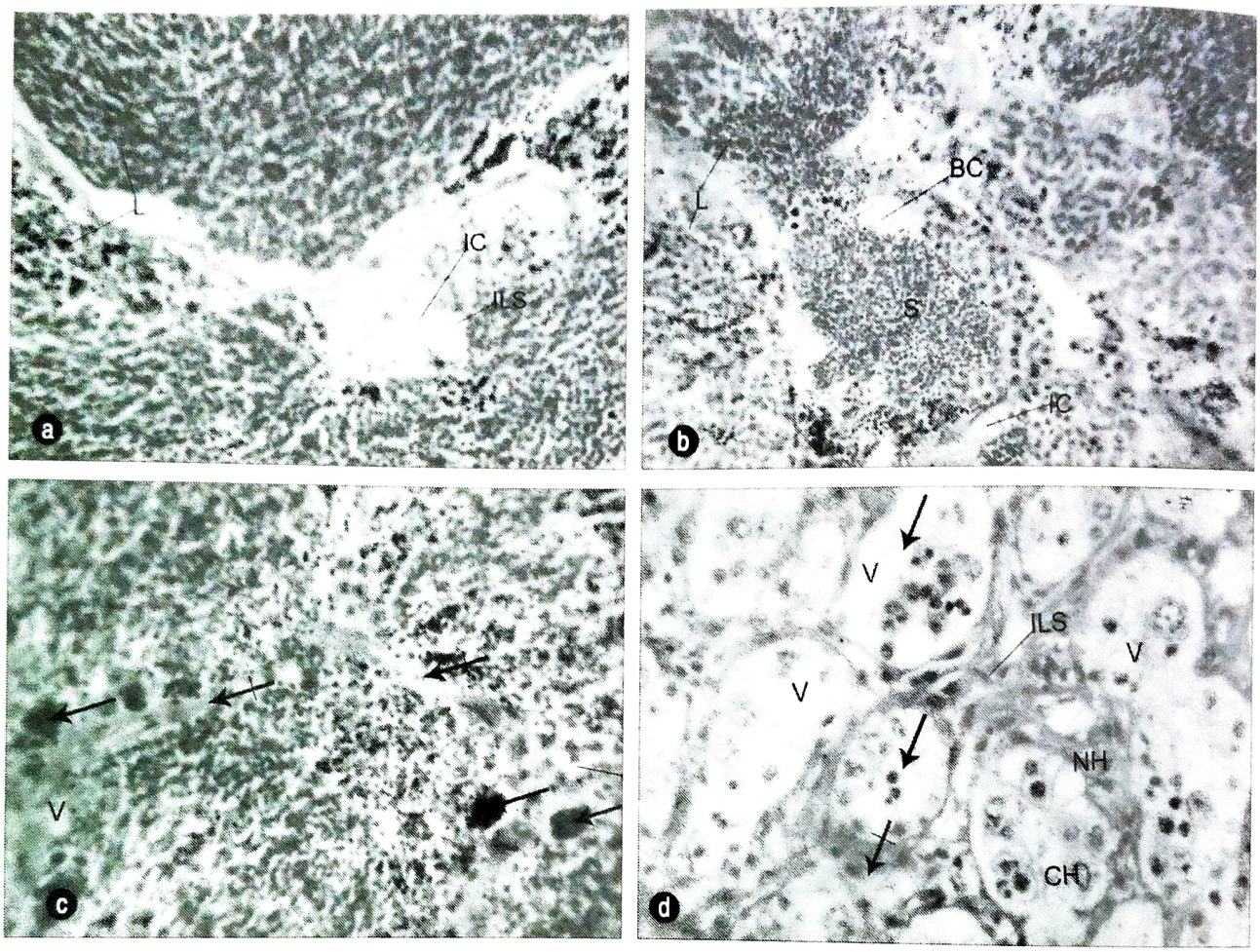

PLATE I: (a) – (c). Photomicrograph of the cross section of the testis of *Heteropneustes fossilis* at control (a-b) and at 15 days of 2, 4-D treatment (c). **(a)** – Showing lobules (L) Containing spermatogonia, spermatocytes, spermatids & sperms. The interlobular space (ILS) contains connective tissue, blood capillaries and interstitial cells (IC). (HE) X 400.Fig **(b)** – Showing clear differentiation of various stages of spermatogenesis in different lobules (L). Also note Blood cells (BC) and interstitial cells (IC) in interlobular space (ILS). (HE) X 400.**(c)** - Showing Clumping of Spermatozoa (arrow). Also note vacuolization (V) and thinning of lobular wall (barred arrow).**(d)** - Photomicrograph of the cross section of the testis of *Heteropneustes fossilis* at 30d exposures of 2, 4-D showing disintegrated cells (arrow) in lobules. Also note disintegration of interstitial cells (barred arrow). Note cellular hypertrophy (CH) and nuclear hypertrophy (NH). (HE, 30 days) x 400

Paraffin sections were cut at 5 μm thick and stained with Ehrich's haematoxyline/Eosin (H/E) for routine histopathological analysis.

Results

Normal Testis: The testis of *Heteropneustes fossilis* are paired structures situated on either side of the body and are attached to the abdominal wall with the help of mesorchium. Histologically the testis reveals lobules of various shapes and sizes which are attached together by thin connective tissue. The interlobular space contains connective tissue, blood capillaries and interstitial cells (Plate Ia,b). As the experiment was conducted during the prespawning phase, the lobules displayed prominent and active spermatogenesis and all the seven kinds of cells *i.e.* sperm mother cells, primary spermatogonia, secondary spermatogonia, primary spermatocyte, secondary spermatocyte, spermatids and sperms could be seen (Plate Ia). The external connective tissue covering sent a number of septa deep into the organ dividing it into lobes and lobules. (Plate Ia). A few blood vessels and capillaries perineated into different regions of the testis. Separated by the intralobular stromal fibres were several nests of prespermiogenic germ cells and vast masses of spermatogonia, spermatocytes, spermatids, and sperms (Plate Ia). In fact, a significant area of all the cross sections of the testis was occupied by the sperms that filled several lobules. This preponderance of the mature gametes was found to be the most characteristics features of the control testis. The extremely large number of sperms rendered the other germinal cell types quite inconspicuous, moreover, the vas deferens was also compactly packed with sperms because of which the duct was visualized only with difficulty. The control testis manifested very active spermatogenic activity. The vas difference was lined by columnar epithelium supported by prominent fibrous connective tissue. Several blood vessels were associated with the fibrosa of the vas deferens.

2, 4 - D Exposure:

15 Days Exposure: Microscopic examination after 15 days exposure showed clumping of spermatozoa at some places (Plate Ic). Vacuolization is also observed in lobules at this exposure. (Plate Ic). There is thinning in germinal epithelium which shows breaking at some places. Thinning in lobular wall is also noticed.

30 Days Exposure: More wear and tear is observed in germinal epithelium, decrease in size of lobules in more marked (Plate Ic). Lobules appear misshaped and distaste, Intensive vacuolization is recorded (Plate Ic). Interlobular space is more pronounced and filled with connective tissue and blood capillaries. Interstitial cells show sign of disintegration (Fig.36). Cellular and nuclear hypertrophy is observed. All kinds of cells get degenerated and appear quite less in number. (Fig.36)

45 Days Exposure: High degree of nuclear degeneration, cytoplasmic clumping and vacuolization is observed. Extensive necrosis and disintegration of lobular boundary cells in the testis have been observed. Interstitial cell show sign of inactivity. Extensive necrosis is observed in spermatogenesis..

90 Days Exposure: Extensive necrosis is discernible in spermatogenesis. Only spermatozoa are seen mostly clumped. Most of the cellular stages are disintegrated as indicated by vacuolization in cytoplasm and pyknosis in nuclei.

Discussion

Marked histological changes were encountered in the testes of *H.fossilis* following herbicide poisoning. The testicular lobules exhibited degenerated spermatogonia and spermatocytes. Similar degenerative changes have also been reported from fish exposed to other toxicants (Pundir and Saxena 1980, Shukla and Pandey, 1984 b, Nath, 1985). Pronounced degeneration of Poecilia reticulata and Oryzias latipes spermatocytes was observed by Wester (1991) when the fish were exposed to BHC and methyl mercury. Ram and Sathyanessan (1987) observed deleterious changes in the spermatogenetic element due to ammonium sulphate poisoning to Channau punctatus and suggested that these changes are altered by direct action on testis itself or indirectly via hypothalamic pituitary-testicular axis in his species. Jyothi and Narayan (1999) studied the toxic effects of carbaryl on the gonads of freshwater fish. Clarias batrachus, and reported reduction in GSI, cessation of spermatogenesis and thickening of basement membrane. The degenerative changes could either be on account of a direct toxic action of the environmental poison or due to an indirect effect via the hypothalamic pituitary-testicular axis as suggested by Ram and Sathyanesan (1987). The interstitial cells of herbicides treated *H.fossilis* exhibited abnormal shapes with damaged cytoplasm and nucleus.

There is scanty information on the deleterious effects of chemical pollutants on these steroid-producing cellular sites in teleost fish. Pundir and Saxena (1980) and Shukla and Pandey (1984b) associate cytonuclear changes in interstitial cells with decreased nucleic acid metabolism. Bagchi *et.al.*, (1990) have observed inhibition of steroidogenic enzyme in Clarias batrachus after quinalphos intoxication, suggesting suppressed function of interstitial cells. Moreover the steriodogenic cells (Interstitial leyding cells) show a significant reduction ($P < 0.01$) in their diameter during the preparatory and mature phases. The interstitial cells of herbicide 2,4-D treated H.fossilis exhibited cytolysis, pyknosis and necrosis pointing to their reduced activity and consequent alteration of spermatogenesis. Active spermatogenesis and formation of sperm were observed in testes from control fish whereas in the treated group only secondary spermatogonia and spermatocytes filled the tubular lumen. Necrotic areas were also evident in these testes. The interstitial cells of the control fish showed activation whereas those of the experimental were exhibiting signs of inactivity. Extensive necrosis in the spermatogenic and interstitial cells are known to be indicative of impaired gonadotrophic activity (Fox and Fox, 1967).

Breaking and dissolution of the lobular wall in the testis of *Heteropneustes fossilis* indicates a degenerative impact of the 2,4-D on this tissue. Similar degenerative changes recorded in certain mammals treated with lindane. DDT, ethyl and methyl parathion and manganese (Dikshith and Datta, 1972; Dikshith, 1973; Seth *et al.*, 1973) are in conformity with our observation in the fish. Sanglang and Halloran (1976), studied the adverse effects of cadmium on the testis of the brook trout and noticed extensive necrosis and reduced lipid content evident from weak Sudanophilic reactions in the lobule boundary cells of his fish. Based on in vitro studies also, there authors have further suggested an alteration in the steroid production in the cadmium treated testis. Minimum charges in there cells during the spawning and post-spawning phases of the testicular cycle of *Colisa fasiciatus* may well be correlated with the attainment of final maturity and consequent ebb of the activity during those places. Cytologists have reported that chemical pollutants inhibit nuclei acid content (RNA-DNA) by generating various chromosomal abnormalities (Palmer, *et al.* 1972; Dikshit, 1973).

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