Bioscience Discovery, 11(4):262-267, Oct - 2020

Open Access, Peer Reviewed, Referred Research Journal

Print & Online available on https://jbsd.in
ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)

Research Article

Histological Changes Induced by Dimethoate in the Testis of Freshwater fish *Puntius ticto* (Ham)

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Article Info

Received: 01-06-2020,
Revised: 22-08-2020,
Accepted: 02-09-2020

Keywords: Histology, Acute and chronic toxicity, Dimethoate and *Puntius ticto*

Abstract

Histological changes induced by dimethoate on the testis of *Puntius ticto* (Ham) were studied. *Puntius ticto* a freshwater fish exposed to lethal (5.012ppm) and two sublethal (2.506 and 1.253ppm) concentration of dimethoate for acute (four days) and chronic (sixty days) exposure. Marked degenerative changes in the testis of *Puntius ticto* were observed during short and long-term exposure to dimethoate. Short-term exposure shows that there was an apparent degeneration in the lobules exhibiting collapse or rupture. Germinal epithelial layer was ruptured and epithelial cells showed swollen portion at some places. Large interlobular spaces were observed. There was necrosis and vacuolation in interstitial Leydig's cells and connective tissue was also observed. Chronic exposure results showed that lobules are highly vacuolated in 2.506 ppm whereas less vacuolation was observed in the lobules at 1.253 ppm exposure which may be due to reduced spermatogenic activity. Degeneration and necrosis were observed in certain places of lobules to both the sub-lethal concentrations. The severity of damage in the testis was found to be dose dependent.

INTRODUTION

Wide use of pesticides in agriculture to control the pests has indirectly created the problems of pollution of aquatic ecosystem. These chemicals are also injurious to non-target organisms like fish. These pesticides enter freshwater resources like ponds, rivers and lakes by various means and ultimately contaminate the water. These pesticides find their way into the body of the aquatic fauna by means of gills, oral membrane, gastro-intestinal mucosa and from general body surface into circulation. These pesticides reach different organs of the body and considerable amount of it is deposited in the sub –dermal fat and other tissues. Due to accumulation of these pesticides in tissues produces many physiological, histological and biochemical changes in the fishes and freshwater fauna by influencing the activities of several enzymes and metabolites (Nagarathnamma & Ramamurthi, 1982). Fish health may thus reflect, and give a good indication of the health status of a specific aquatic ecosystem. Therefore, it is necessary to study in detail the histopathological alterations or changes in structure produced by pesticides in different organs of fishes and thoroughly investigate them in order to assess the extent of damage.

Histopathology deals with the study of pathological changes induced in the microscopic structure of body tissues. Any peculiar alteration of cell may indicate the presence of disease or toxic substance. In fishes it is observed that the external organs are affected due to the toxic chemicals causing loss of equilibrium, increase in opercular movements, to and fro, irregular or vertical movements, finally leading to death. This may be attributed to the significant damage to the internal organs. Histopathological changes are used as

biomarkers to evaluate the overall health of fish exposed to contaminants (Adams, 2002). Thus, it gives useful data concerning tissue changes prior to external manifestations. Hence the present study deals with the effect of dimethoate pesticide on the histopathological changes in the testis of *Puntius ticto* (Ham) under acute and chronic exposure.

MATERIAL AND METHODS

The freshwater fish *Puntius ticto* were collected from the freshwater sources around Aurangabad city. They were acclimatized in aged, dechlorinated and well aerated water for two weeks in the laboratory. During acclimatization they were fed on alternate days with pieces of live earthworms. The LC_{50} values are determined by following the guidelines given by committee of toxicity tests with aquatic organism (Annon, 1975) and Probit Analysis Method (Finney, 1971). The 20 healthy and acclimated fishes showing normal activity were exposed to lethal concentration (5.012ppm) for 96h (Acute Study) and sublethal concentrations (2.506ppm and 1.253ppm) for 60 days (Chronic Study). Simultaneously a control group of healthy fishes were maintained under identical conditions. After commencement of exposure period fishes were killed by decapitation and testes are removed and fixed in Bouins fluid for 24h and processed according to standard procedure of routine microtechnique. For staining double stain method was followed by using Haematoxylin and Eosin and mounting was done in DPX.

RESULTS AND DISCUSSION

Histology of Testis:

The testes of *Puntius ticto* are paired elongated structures which remain suspended with mesorchium. Each testis is bean shaped structure, composed of radially arranged lobules bound on the coelomic surface covered by a thin visceral peritoneum. Between the basal lamina of adjacent lobules is the inter-lobular septum. The seminiferous lobules are lined by germinal epithelium. Each lobule in section is associated with germ cells. Germinal cells in various stages of differentiation are contained within each lobule. Different stages of spermatogenesis viz. sperm mother cells, spermatogonia, primary and secondary spermatocytes, spermatids and sperms were observed inside the lobule. The interstitial cells appear to communicate with each other through interlobular connective tissue corridors.

Spermatogonia are the only type of germ cells present in an immature testis. Each spermatogonium is globular or ovoid with distinct round nucleus. The interstitial cells can be identified from the connective tissue cells during this stage. Maturing testis has actively dividing germ cells. In a seminiferous tubule several nests of multiplying germs cells are present. Each spermatogonium has cytoplasm, prominent nucleus and a nucleolus. In mature testis, lumen of the tubule is wide and filled with sperms. The actively dividing nests of germ cells are much less in number than those in the preceding stage.

Histopathology of Testis

Marked degenerative changes in the testis of *Puntius ticto* were observed during short and long-term exposure to dimethoate. Short-term exposure shows that there was an apparent degeneration in the lobules exhibiting collapse or rupture. Germinal epithelial layer was ruptured and epithelial cells showed swollen portion at some places. Large interlobular spaces were observed. There was necrosis and vacuolation in interstitial Leydig's cells and connective tissue was also observed.

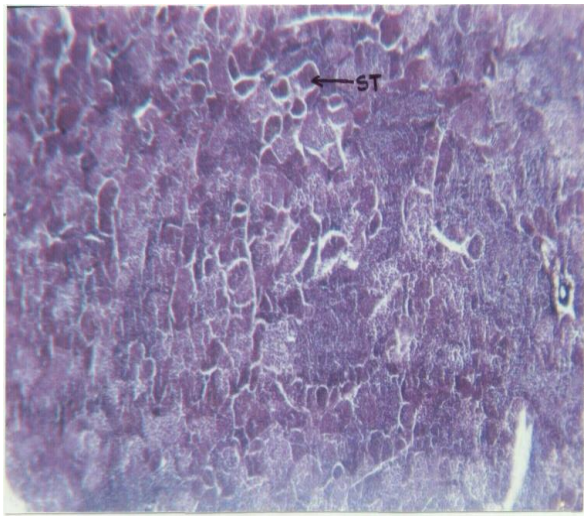
Chronic exposure results showed that lobules are highly vacuolated in 2.506 ppm whereas less vacuolation was observed in the lobules at 1.253 ppm exposure which may be due to reduced spermatogenic activity. Degeneration and necrosis was observed in certain places of lobules to both the sub-lethal concentrations. Flattened germinal epithelial layer was observed at certain places, in both the sub-lethal concentrations during chronic exposure. The severity of damage in the testis was found to be dose dependent.

Cope (1966) reported that the herbicide at sublethal levels to blue gills cause degenerative lesions in liver and testis. The degenerative changes in seminiferous tubules, enlarged interstitium and haemorrhage in inter-tubular area in albino rats exposed to pesticides have been reported by Dutta and Dikshit (1973), Nigam *et al.,* (1979) and Baronia and Sahai (1993). Ahsan and Ahsan (1974) conducted that cadmium chloride blocked the spermatogenic activity at the secondary spermatocytes level in *Clarias batrachus* and the changes were pycnosis, vacuolation and degeneration in the spermatogenesis. Eroschenko and Wilson (1974) reported that the testes from maturing and fully grown CD fed quail were severely affected. Generally, there was a reduction of the germinal epithelium thickness and number of

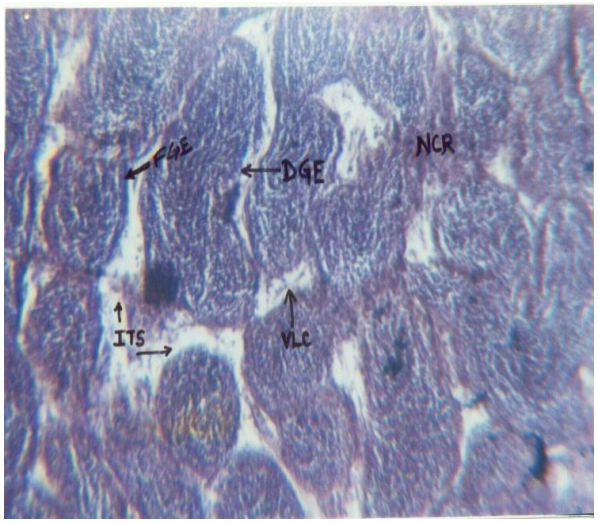
spermatozoa. Sangalang and Halloran (1973) also studied the effect of Cd (25 ppm for 24 hrs) in brook trout testis and found necrosis and disintegration in the lobule boundary cells and they reported that Cd directly affects the testicular steroidogenesis. Similar results were observed during present study

Pandey and Shukla (1982) studied the effect of BHC in *Tilapia mossambica* testis and found vacuolated cells, necrosis in the seminiferous lobules, atrophied interstitial cells and thickened sperm duct wall. They also reported that in 2 ppm treatment, very thin and broken lobular connective tissue septum, seminiferous lobules lost their shape and most of the cells in their inner core atrophied. 4 ppm BHC treatment showed more pronounced changes, where primary spermatocytes revealed hypertrophic changes with distinct cytoplasmic vacuolation. Shukla (1982) reported that DDT, BHC and Malathion inhibited spermatogenesis at spermatid level in *Tilapia mossambica.* Sehgal (1982) studied the effect of certain pollutants on the histological changes in testis of guppy *Lebistes reticulates*. He observed that BHC in 1 ppm concentration CuSO4 at 0.5 ppm concentration in a treatment period of 31 days caused reduction in number and vacuolation in spermatogonal cells, primary and secondary spermatocytes and spermatids. Nagabhushanam *et al.,* (1983) found that acute exposure of malathion, dimecron and monocrotophos caused pronounced changes in the gonad of a freshwater prawn, *Macrobrachium lamerrii*. Sehgal *et al.,* (1984) studied the comparative effect of heavy metallic salts on the on the testis of *Lebistes reticulates* and observed most prominent changes on 30 days of exposure, reduction in number of different spermatogenic cysts was observed. $CdCl_2$ highly affected the spermatogenic nest and spermatocytes; while $CuSO_4$ affected the spermatid and sperm cysts. They also observed vacuolation in spermatocytes and increased atretic spermatophores in both heavy metals. Shukla and Pandey (1984) studied the carbamide induced histological alterations during different phases of the testicular cycle of a freshwater perch *Colisa fasciatus.* They found the abnormal lobular architecture, dissolution of germinal epithelium, prominent vacuolization and necrosis during the preparatory and mature phase. Muley and Mane (1987) studied the effect of cythion-malathion on the gonads of Lamellibranch *L. cirrianus* and *L. marginalis* and observed that pesticide cause severe damage in both species and they also affect the free mature gametes in the lumen of the follicles and appeared to stop the growth of the germ cells and development of the sex cells. Khillare and Wagh (1989) studied the effect of endosulfan, malathion and sevin on the testis of *Barbus stigma* and observed endosulfan brought severe damage. Degenerations were found in the spermatocytes, spermatids and interstitial cells. Seminiferous tubules were highly affected. Epithelial cells were also ruptured and the blood vessels were found congested. In malathion toxicity, they observed seminiferous tubules were ruptured and interstitial cells showed degenerative appearance. Spermatocytes were not greatly affected and the epithelial cells showed swollen portion at some places. In sevin toxicity, they observed ruptured tubules and shrunken interstitial cells.

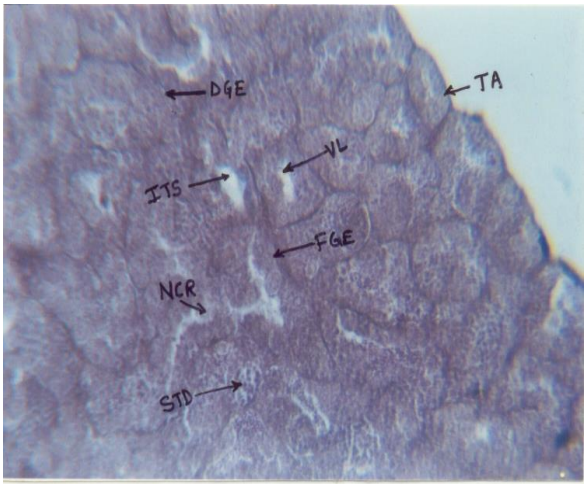
Pundir and Saxena (1990) studied seasonal changes in the testes of *Puntius ticto* under heavy metal toxicity. They found degenerative changes during all phases of testicular cycle after sub-lethal exposure of heavy metal. Testes of cadmium treated fishes in the early stage of maturation exhibited vacuolization in spermatogonia and spermatocytes. The connective tissue septa between the lobules ruptured and interstitial cells atrophied. In maturation phase, they observed abnormal architecture of lobules. Nath and Kumar (1990) studied effect of nickel on testis of *Colisa fasciatus.* They observed marked degenerative alteration. There was general disorganization in the testicular lobules, degeneration of lobules exhibited collapse or rupture and blood vessels were congested. Reduction in the spermatogenesis and degeneration of the lobules evidenced in *C. fasciatus* have been reported after treatment of the fish with cadmium (Wani and Latey, 1982) and Zinc, Copper and lead (Kumar and Pant, 1984,). Begchi *et al.,* (1990) studied the effect of quinalphos on testis of *Clarias batrachus* and observed significant decrease in the weight of testes, degenerative changes in seminiferous epithelium and shrinkage of seminiferous epithelium. Shrinkage of seminiferous tubular diameter further confirms the possible diminution of testicular steroidogenesis and androgen production. Srivastava and Srivastava (1994) observed extensive damage in the testes of *Heteropneustes fossilis* on exposure to both acute as well as long-term sub-acute to chlordecone. They observed flattened and reduced seminiferous tubules, degenerated and desquamated germinal epithelium and thin inter-tubular connective tissue.



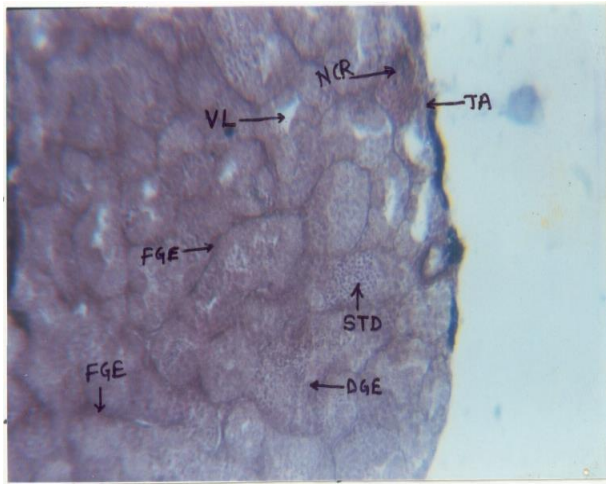
A)T.S. of testis of *Puntius ticto* (Control)
Haemotoxylin/Eosin 100X
ST-Seminiferous tubule



B)T.S. of testis of *Puntius ticto* after 5.012 ppm exposureto dimethoate, Haematoxylin/Eosin 400X
DGE-Degenerated germinal epithelium
NCR-Necrosis
FGE-Flattened germinal epithelium
VLC-Vacuolated Leydig cells
ITS-Inter-tubular space



C)T.S. of testis of *Puntius ticto* after 2.506 ppm exposure to dimethoate, Haematoxylin/Eosin 400X
DGE-Degenerated epithelium
FGE-Flattened germinal epithelium
ITS-Inter--tubular space
NCR-Necrosis,
STD -Spermatids
TA-Tunica albuginea
VL-Vacuolated testicular lumen



D)T.S. of testis of *Puntius ticto* after 5.012 ppm exposure to dimethoate, Haematoxylin/Eosin 400X
DGE-Degenerated germinal epithelium
FGE-Flattened germinal epithelium
NCR-Necrosis,
STD-Spermatids
TA-Tunica albuginea
VL-Vacuolated testicular lumen

Spermatids and sperms showed cytolysis and interstitial tissue showed atrophied and vacuolized Leydig cells. Awari (1991) reported several structural changes in the testis of *Anabus ranga* subjected to $CdCl_2$ treatment.

They also observed distortion of spermatids and pycnosis of spermatogenic cells. Sakthivel and Gaikwad (2001) exposed male *Gambusia affinis* to sub-lethal concentration of dimecron and observed excessive degeneration and vacuolization of seminiferous tubules, distortion of spermatids and pycnosis of spermatogenic cells. Kalsoom *et al.,* (2005) observed that histological examination revealed disintegration of some of the spermatogenic cells within the cysts of some lobules in *Cyprinion watsoni*. In addition, treatment with 1 ppb endosulfan leads to loosely arranged cysts and lobules i.e., the interlobular space increased.

Kumar *et al.* (2007) observed gross damage of germinal epithelium, inflammatory response, interlobular vacuolations and contraction and condensation in the cells of tubules under all sets of intoxication. They also stated that the effect is exposure dependent and concentration mediated. Similar findings were also observed by Katti and Sathyanesan (1985), Kinberg *et al.* (2000) and Kumar *et al.,* (2007). Banayi *et al.,* (2009) found degeneration of seminiferous tubules, atrophy and vacuolations in testicular tissues in diazinon treated fish *Cyprinus carpio* and also stated that these changes were time and dose dependent**.** Singh *et al.,* (2015) studied toxicological effects of Lambda-cyhalothrin on liver, kidney and testis of Indian Catfish *Clarias batrachus* and found diminished sperms, diminished stroma empty seminiferous tubules, degenerative and Leydig cells. Fakhar *et al.* (2017) observed increase in interlobular areas and clumping patterns in spermatocytes. Finally, it can be concluded that exposure to dimethoate pesticide can results in degenerated germinal layer, necrosis, formation of inter-tubular space, degenerated seminiferous tubules, vacuolated Leydig cells and vacuolated tubular lumen may be leads to decreased fertility potential in *P. ticto*

ACKNOWLEDGEMENT

I am thankful to the Head, Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University who allowed me all necessary facilities to do my work. I am grateful to the Principal, Smt. Meenalben Mehta College Panchagani Ta. Mahabaleshwar, Dist. Satara for their kind help and inspiration

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How to cite this article

Ganeshwade R. M., 2020. Histological Changes Induced by Dimethoate in the Testis of Freshwater fish *Puntius ticto* (Ham). *Bioscience Discovery*, **11**(4):262-267.

Google Scholar citation: https://scholar.google.co.in/citations?user=vPzEyC8AAAAJ&hl=en