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EFFECT OF 2,4-DICHLOROPHENOXY ACETIC ACID (2,4-D) ON OVARY OF A FRESH WATER CAT FISH, *HETEROPNUESTES FOSSILIS*

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ABSTRACT – Effect of sub-lethal concentrations of 2,4-D on ovary of a fresh water catfish *Heteropnuestes fossilis* has been studied. Ovary of *H.fossilis* exposed to 2,4-D revealed pronounced histopathological abnormalities. The decrease is number of oogonium suggests that 2,4-D causes arrest of oogonium formation from germinal epithelium. Further numerical increase of atretic follicles also suggests that the mature and maturing follicles have undergone degeneration. The increase in interfollicular spaces has been as markedly observed.

Keywords : 2,4-D, ovary, fresh water catfish, *Heteropnuestes fossilis*.

INTRODUCTION

2,4-D is 2,4-Dichlorophenoxyacetic acid and is used in herbicides. 2,4-D, a chlorinated phenoxy compound functions as a systemic used in cultivated agriculture and posture and large land applications, forest management, home and garden situations and for the control of aquatic vegetation. Unfortunately the herbicide does not affect the target weeds alone. It can cause low growth rates, reproductive problems, change in behaviour and death in non target species. This chemical is carried by surface run off into local ponds and river system affecting the aquatic organisms including fish.

Various Workers have observed the effect of different pollutants on fish ovary. However, the study on effect of herbicide, 2, 4-D on fish ovary is sporadic and scanty. Kulreshtha and Aronra (1984) studied the effect of carbaryl and endosulfan on oocyte formation of *Channa striatus*. Pandey (1988) reported the impact of endosulfan on dynamics of oocyte development in *Colisa fasciatus*. Dutta *et al* (1992) observed sub-lethal effect of malathion on the ovary of *Heteropneustes fosslis*. Gormley and Teather (2003) made study of reproductive effect of endosulfan in Japanese medaka (*Oryzias latipes*). Datta and Maxwell (2003) observed effect of diazinon on ovary of *Lepomis macrochirus*. Daesick *et al* (2004) investigated the effect of endosulfan on breeding and nonbreeding female mosquito fish. Datta and Dalal (2008) studied the effect of endosulfan on ovary of *Lepomis macrochirus*.

The present study has been undertaken to examine the effect of the herbicide, 2, 4-D on ovary of a fresh water cat fish, *Heteropneustes fossilis*.

Heteropneus 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 bolders 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 family Heteropneustidae and order siluriformes.

MATERIALS AND METHODS

Live specimens of *Hetoropneustes fossilis* (approx, length 15 + 3 cm and wt 50 + 10g) were collected from local ponds and river Gomati at Jaunpur city (U.P.) and were acclimated to optimum lab conditions for 15 days. Fish during acclimatization were fed with minced goat liver on alternate days, Fishes were exposed to sublethal concentration (0.6 mg/litre) of 2,4-D. Fishes were cold anaesthetized following Mittal and Whiter (1978) and ovary from both sides of fishes were excised, rinsed in saline water and were fixed in 10% neutral formation and Bouins'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. Paraffin sections were cut at 5µm thick and stained with Ehrlich's haemeatoxylin/ Eosin (H&E) to study structure of ovary.

OBSERVATION

Control :

The ovaries of *H fossilis* are elongated paired structures and are attached to the body wall with the help

of mesovarium. The ovarian wall is fairly thick and vascular and consists of three layers, outer most thin peritoneum, a middle thicker tunica albuginea and inner most layer, the germinal epithelium which project into ovacoel in the form of ovigerous lamellae. These ovigerous lamellae are the seat for the development of oocytes which are visible in various stages of development. Oogonia are in clusters and originate from the germinal epithelium. The developing oocytes, are visible in different stages (oocyte I, oocyte II, oocyte III and oocyte IV) of development. Yolk vesicles (YV) and yolk granules (YG) present periphery of the Ooplasm and extra vesicular ooplasm. *Zona radiata* (ZR) visible between ooplasm and the follicular layer. Ovigerous lamellae (OL) and follicular lining are intact as well.

Herbicide 2,4-D Treatment -

After 15 days exposure atretic oocytes are seen abundantly. Nucleoli are not clearly visible in any stage of oocyte, Ovigerous lamellae appear loose. Zona radiata in mature oocytes appear separated at some places.

After 30 days of exposure oocyte-I and oocyte-III are most abundant. Oocytes-II and Oocytes-IV are less in number as compared to oocyte-I and oocyte-III. Atretic oocytes (AO) are rarely observed. Ovigerous wall and lamellae are quite thick.

After 45 days exposure, nuclear retraction and cytoplasmic retraction is observed in almost all stages of oocytes. Ovigerous lamellae also show sign of disintegration. Sign of disintegration is also seen in ovigerous wall, germinal epithelium and oogonial cells.

After 60 days treatment, growth of oocytes of stage I, Stage II and Stage III found to be retarded. Stroma found to be extremely degenerated. Ovarian wall and ovigerous lamellae are found broken. In most cases stage IV oocytes are found expelled from zona radiata and follicular layer of oocytes IV found separating from the oocytes. In some cases Stage IV oocytes look almost empty with degeneration of nuclear material and ooplasmic material.

After 90 days, Ovarian tissue exposed to 2,4-D increasing length of time. i.e. 90 days had increasing amount of damaged cells. Ovigerous lamellae and follicular lining were seen free floating near the oocytes, no longer attached. Necrosis or loss of genetic material of the neuclei are observed. The nculear membrane, zona radiata and follicular layer are found damaged with release of nuclear content in stroma. Karyoplasmic clumping, cytoplasmic retraction and vacuolization in ooplasm is evident. Oocytes of all the stages were found degenerated.

DISCUSSION

Ovary of *H.fossilis* exposed to 2,4-D revealed pronounced histopathological abnormalities. The effect of 2,4-D exposure on fish ovary is sporadic and scanty. The decrease in number of oogonium suggests that 2,4-D causes arrest of oogonium formation from germinal epithelium. Further numerical increase of atretic follicles also suggests that the mature and maturing follicles have undergone degeneration. The increase in interfollicular spaces, as markedly observed in the present study, has also been observed by many earlier workers in fish exposed to various pollutants (Mukherjee, 1975; Saxena and Arora, 1984; Shukla and Pandey, 1984a; 1984c; Shukla, 1995). Similarly, the presently observed increase in the number of atretic oocytes has previously been reported in fish species exposed to other toxicants like thiourea (Mukherjee, 1975), heavy metals (Pant, 1982; Wani and Latey, 1982; Nath, 1985), pesticides (Saxena and Garg, 1978; Shukla, 1995) and industrial wastes. (Prasad, 1984). Pandey, 1970 had observed effect of thiourea on ovary of juvenile guppy. Ovary of *H. fossilis* also dispaly histological alteration and increased interfollicular spaces when treated with fenvalerate (Srivastava, 2000). Ovarian recrudescence has been reported in *Channa punctatus* and *Clarias batrachus* after long term treatment with $HgCl_2$, Cythion, ammonium sulphate and Emisan 6 (Ram and Sahyanesan, 1983, 1984, 1985, 1986; Kirubagaran and Joy, 1988). Jyothi and Narayan (1999) have reported interfollicular odema accompanying ovarian recrudescence in *Clarias batrachus* exposed to carbaryl. The degenerative changes observed in *H.fossilis* after intoxication with Dimethoate and fenvalerate pesticides leading ultimately to atresia formation may be attributed either to a lowered level of gonadotropin secretion (Srivastava *et al*, 1998 and Srivastava, 2000) or decreased nucleic acid metabolism (Shukla and Pandey, 1983, 1984a, 1984c).

Saxena and Garge (1978) observed effect of insecticidal pollution on ovary of *Channa punctatus*. Shukla and Pandey (1984a) have studied effect of Arsenic on ovary of *Colisa fasciatus*. Mukharjee (1975) demonstrated effect of thiourea on ovary of *Heteropnuestes fossilis*. Wani & Latey (1982) have studied effect of cadmium on gonads of *Garra mullys*. Ram and Sathyanesan (1986) observed effect of mercurial fungicide on gonadal development of *Channa punctatus*. Ram & Sathyasen (1984) showed effect of ammonium sulphate on ovary of *Channa punctatus*.

Ellis and Roberts (1978) reported severe oedema in the ovary of selenium poisoned catfish, *Ictalurous punctatus*. Saxena and Garg (1978) also reported that

Fig. 1

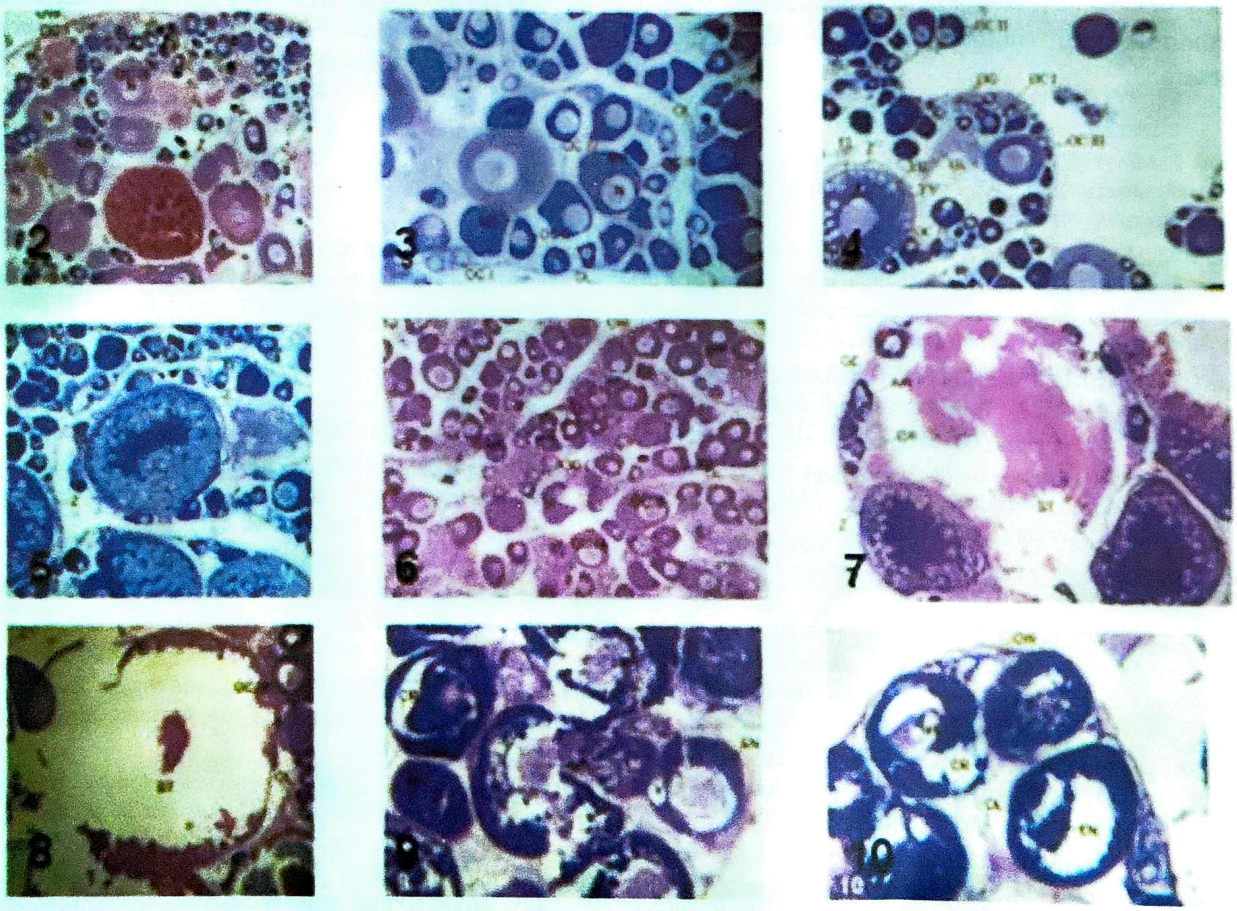

Fig. 1 : *Heteropnuestes fossilis*.

Fig. 2 - 4 : Photomicrograph of the cross section of the ovary of control *Heteropneustes fossilis*.

Fig. 2 : Showing each stage of oocyte (oocyte I, II, III and IV) present in abundance. A large nucleus (N) can be seen in oocyte III cells. The ovarian wall is thick and intact. Atretic oocyte (AO) are also noticed. (HE) X 400.

Fig. 3 : Showing thick ovigerous lamellae and oocytes in different stages. Provitelline nucleoli (PN) can be noticed. Also note Atretic oocyte (AO). (HE) X 400.

Fig. 4 : Showing provitelline nucleoli (PN) and Euvitelline nucleoli (EU). The follicular lining (FL) and Zona radiata (Z) are district and intact. Numerous yolky vesicles (YV) and yolk granules (YG) and present in mature oocyte IV cells. (HE) X 400.

Fig (5) – (7) : Photomicrograph of the cross section of the ovary of *Heteropneustes fossilis* at different exposures of 2, 4-D .

Fig 5 : Showing abundance of atretic oocytes (AO). Note loose ovigerous lamellae (OL). Zona radiata (Z) appear separated in mature oocytes. (HE, 15 days) X 400.

Fig. 6 : Showing abundance of oocyte I and oocyte III cells. Note thick ovarian wall (OW) and ovigerous lemellae (OL). (HE, 30 days) X 400.

Fig. 7 : Showing nuclear retraction (NR) and cytoplasmic retraction (CR) in oocytes. Note disintegrated stroma (ST) and sepration of Zona radiata (Z) from the mature oocyte. Also note disintegrated germinal epithelium (GE). (HE, 45 days) X 400.

Fig. 8 : Showing retardation of oocyte I, II & III. Note extreme degeneration of ovarian wall and ovigerous limellae. (HE, 60 days) X 400.

Fig. 9 : Showing most several damage. Note necrosis, Cytoplasmic retraction (CR), nuclear retraction (NR), expelled nucleus (EN), vacuolization (V). (HE, 90 days) X 400.

Fig. 10 : Showing necrosis or loss of genetic material. Note damaged nuclear membrane, zona radiata & follicular layer. Also note damage of oocytes of all stages. (HE, 90 days) X 400.

carbaryl and fonitrothion treatment arrested ovarian recrudescence to various degrees in *Channa punctatus*. However, carbaryl was more effective and did not allow the appearance of stage IIIrd oocytes and in the number of atretic follicles increased. The sub-lethal concentration of 2,4-D did not produce marked histological changes in the ovary of *Colisa chuna* in early exposures. Our finding including prominent follicular spaces, reduction in the development of mature oocytes, restricted deposition of yolk globules, increased atretic structures and stromal haemorrhage are in conformity with the finding of above workers.

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