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Histopathological Impact of Organophosphorus Pesticide, Malathion on the Ovary of Freshwater Fish *Tilapia niloticus* (Linnaeus, 1758)

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Abstract

Malathion is an organophosphorus class of pesticide widely used as an insecticide in the agricultural field of Kerala. The present investigation was focused on the effect of sublethal concentration (6.0 ppm) malathion in the ovary of the freshwater teleost, *Tilapia niloticus* for thirty days with parallel untreated control. Exposure dependent alternation in ovary and histology is reported in a period of exposure time such as reducing the size of mature oocytes, degeneration of epithelial cells, destruction follicular epithelium fragmented ova, necroses and the complete loss of normal configuration of the ovary.

Keywords: Toxicity, Malathion, Ovary, *Tilapia niloticus*

1. Introduction

Fishes are relatively sensitive to changes in their surrounding environment including an increase in pollution (Muraya *et al*., 2016). Aquatic pollution by pesticide is a serious problem in India and is causing many enormous health problems (Scholz *et al*., 2012). In modern agriculture for crop protection number of pesticides are used, but 0.1% of theses applied pesticides are act on target species and the remaining amount of pesticides persisting and spreading throughout the environment (Macneale *et al*., 2010). Through runoff they reached into the aquatic ecosystem and cause aquatic pollution, it disturbed the ecological balance (Hart and Pimentel, 2002). Pesticide doses that are not lethal to fish may affect their physiology and behaviour, ultimately damage survival and reproduction (Kegley *et al*., 1999). Metabolic disturbances, growth retardation, enzyme inhibition, reduction in the longevity and fecundity of the organisms and some biochemical changes caused by the pesticidal stress (Murthy, 1986). Malathion is an organophosphate compound it is used as an insecticide and widely used in agricultural field and houses to control a wide variety of pests and insects including aphids, beetles, scales and pill bugs and are used in residential landscaping, and public recreation area and in public health pest control programmes. It is an acetylcholinesterase inhibitor and is accumulated in neuromuscular junction and cause muscle twitching and paralysis. Malathion is toxic to aquatic organisms but has relatively low toxicity for birds and mammals but it greatly affects on non-target species. The freshwater fish, *Tilapia niloticus* has greater economic value because it is eaten as a popular food, due to its high nutritional value, taste and low expenses. The present study was undertaken to investigate the detailed histopathological changes induced in the ovary of *T. niloticus* exposed to sublethal concentration of malathion for short term and long term periods.

2. Materials and Methods

Adult (male and female) *T. niloticus*, collected from a local farm, is used for the experiment. Same sized (~14 cm) healthy fishes were introduced into the aquarium for acclimatization under laboratory condition for 10 days. Malathion (Malik insecticide - 50% E.C) purchased from local pesticide shops were used for LC_{50} study. The tests were performed in standard size glass aquariums (two tanks, one control and two experimental). The physico-chemical parameters of experimental water were noticed (Temperature 28±1 °C, DO 7.1 -7.3 mg/l, pH 6.7-7.1). The LC_{50} was determined by Probit analysis method (Finney, 1971). The LC_{50} value of 24 hrs was found to be 14.56 ppm, 48 hrs 12.72 ppm, 72 hrs 12.22 ppm and 96 hrs 10.04 ppm. The sublethal concentration of malathion used for the present experiment was 6.00 ppm. The test fish were kept in the test solution of known concentration for a period extending 30 days. After every 24 hours, fresh test solution was introduced. The experiments were run in replicates and also with control groups. The test and control group fishes were sacrificed in alternate days like 2nd day, 10th day, 15th days and 30th days, the ovary is dissected out, fixed in Bouin's fluid, preserved and processed for histological analysis. Fixed tissues were embedded in paraffin wax, sectioned (5µm) and stained with suitable stains (haematoxylin and eosin) and mounted in DPX (Roberts, 1978). The slides were photographed with Olympus BX-51 and the images were analyzed by Olympus microimage software.

3. Results and Discussion

The ovary of normal teleost fishes is a hollow sac-like organ. The ovarian follicle is an aggregate of ova and epithelial cells. This follicle starts as oogonia or primitive mother cells, which are periodically generated in the germinal epithelial. The oogonia are made up of cells which are beginning to mature to form and produce

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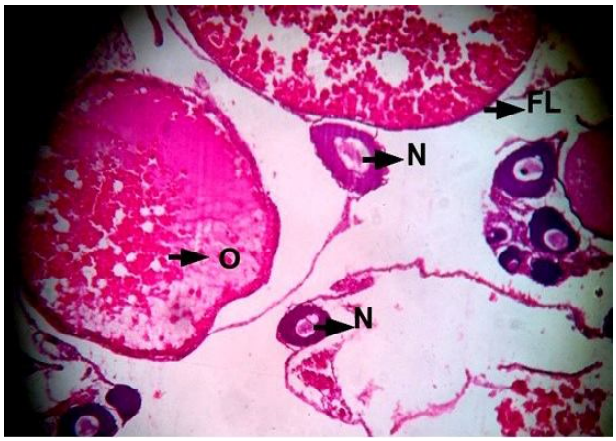

Fig. 1. Ovary of control fish *Tilapia niloticus.* (O -Oocyte, FL- Folicular lining, N -Nucleus)

oocytes. Small epithelial cells form a single layer surrounding the oogonia. The epithelial cells grow as the ovum and are separated by a gradually thickening hyaline capsule. These are responsible for nourishing the ovum and secreting its yolk. The ovarian wall contains numerous blood capillaries, the connective tissues are seen in normal. The germ cells become associated with epithelial cells, the oocytes develop into mature ovum nourished with follicular cells. In the present study, control fish ovary shows normal structure without any histological changes in compared to the experimental fishes (Fig. 1).

Lethal concentration (LC_{50}) is mostly acceptable tool basis for the acute toxicity test. In the present study, LC_{50} value for the treatment 24 hrs, 48 hrs, 72 hrs and 96 hrs was found to be 14.56 ppm, 12.72 ppm, 12.22 ppm and 10.04 ppm respectively. The studies of Shao-Nan (1996) estimated that LC_{50} values range from 0.25 to 15 ppm. Thenmozhi *et al*., (2011) revealed that 96 hrs LC_{50} value of malathion is 15 ppm in *Labeo rohita*. The variation in LC_{50} value of the same pesticide against the same or different species, maybe due to the toxicity or concentration of pesticide, the absorption of pesticide and their accumulation, biotransformation, excretion and it mainly depends on fish body weight, size, exposure time, breathing rate etc. the entering of pesticide in fish severe impairment in vital organs, physiology and the health of the species (Naserabad *et al*., 2015).

Toxicity of malathion is very toxic to fish species. Many authors suggested the effect of pesticide on fish's ovaries (Pandey *et al*., 2005; Wasu *et al*., 2009; Ahmad, 2012; Sharmin *et al*., 2015). In the present study, malathion affected the normal structure of the ovary and showed many structural and degenerative changes. Fishes exposed to sublethal concentration of malathion during 2 days to 30 days of interval shows considerable changes. In acute exposure for 2 days, degeneration of cytoplasm and vacuolisation was observed (Fig. 2A), in 10 days of exposure period shows reduced oocytes and oocyte damages (Fig. 2B). In chronic exposure 15 days shows destruction of follicle, oocyte damage, fragmented ova were observed (Fig. 2C). The long term exposure period 30 days, complete loss of normal configuration of the ovary, necrosis, oocyte damage, retarded follicle cell, fragmented ova with abnormal shape were observed. It also shows some prominent changes of oocytes, the normal structure of ovary reduced and it delays the growth of oocytes follicles (Fig. 2D). Similar observations were made by Kulshrestha and Arora (1984) in *Channa striatus.* Hazarika and Das (1998) observed many structural changes in the ovary of *Heteropneustes fossilis* during 1 ppm, 5 ppm and 10 ppm of BHC exposure and also observed that the BHC exposure dose alter the normal ovary structure of fish. Fragmentation and karyolysis of ova were observed in *Salmo gairdneri* and *Cyprinus carpio* were exposed to Aroclor 12.54 (Shivarajah *et al*., 1978). Srivastava *et al*. (2008) reported devicyprin induced many gonadal impairments in a freshwater fish *Channa punctatus* (Bloch).

Malathion exposure creates prominent damages such as ovary and oocyte damage, cytoplasmic retraction of oocytes, destruction of follicle, broken ovarian wall, extrusion of karyoplasms (Deka and Mahantam, 2012). The higher and lower dose of malathion exhibits severe changes in ovary. Lower dosage of malathion reduces the total ovarian weight and growth reduction, retarded growth of previtellogenic oocyte and the higher dose exposure altering the histology of normal ovary, degeneration of immature oocytes, rupturing of follicular epithelium, necrosis and these findings suggests the changes due to the imbalance of endocrine system (Ramachandra, 2000). Hiran and Maxwell (2003) explain the effect of insecticide diazinon (organophosphorus) on the ovaries of bluegill (*Lepomis macrohirus*) and this exposure create in ovary serious histopathological lesions noticed, adhesion of primary follicle cytoplasmic retraction in oocytes, cytoplasmic degeneration, increased atretic oocyte, oocyte damage, destruction of ovigerous lamellae, increased follicular space, vacuolated cytoplasm, extrusion of karyoplasm and necrosis. These findings suggest that the histopathological changes in the ovary might be a reflection of the disturbance in the endocrine/hormonal imbalance (Ramachandra, 2000).

4. Conclusion

In the present investigation on the effect of malathion on the ovary of freshwater fish *T. niloticus* shows deleterious changes. A considerable degree of alteration in the ovary, reduction in size of mature oocytes along with vacuolization, degeneration of cytoplasm, reduced oocytes, degeneration of epithelial cells were observed. In chronic exposure complete loss of normal configuration of ovary destruction of follicles, oocyte damage and destruction of the ovarian wall, fragmented ova, retarded follicle cell. Long term exposure to organisms to pesticides means continuous health problems for the population. So the human population face high risk by consuming these toxic fishes. This implies that one should take necessary safety precautions in the application of pesticides to protect aquatic life. This study gives an idea of the intensity of the hazardous effect of malathion on fishes.

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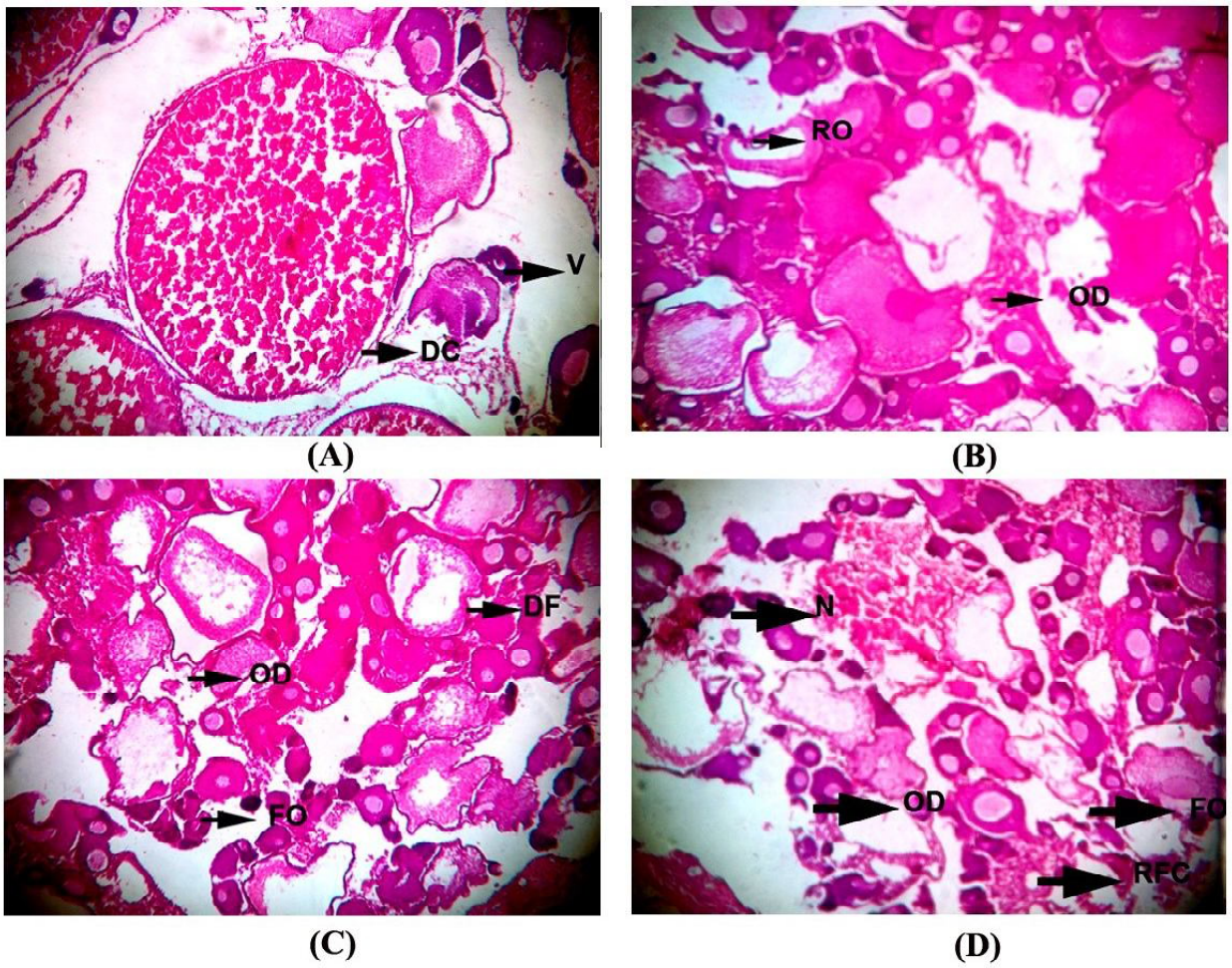

Fig. 2. (**A**) Ovary of 2 days treated fish *Tilapia niloticus* (V -Vacuolation, DC- Degeneration of cytoplasm; (**B**) Ovary of 10 days treated fish *T. niloticus.* (RO- Reduced Oocyte, OD- Oocyte damage); (**C**) Ovary of 15 days treated fish *T. niloticus.* (DF- Destruction of Follicle, OD- Oocyte Damage, FO- Fragmented ova); (**D**) Ovary of 30 days treated fish *T. niloticus.* (N- Necrosis, OD- Oocyte Damage, FO- Fragmented Ova, RFC- Retraded Follicle Cell).

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