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Article

Histoarchitecture changes in the ovary of Stinging catfish, Shing (*Heteropneustes fossilis*) under cypermethrin toxicity

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Abstract: Histoarchitecture effect of cypermethrin (pyrethroids) was investigated in the ovary of Stinging catfish, Shing (*Heteropneustes fossilis*) over a 96 h exposure period as an endpoint of toxicity. The fish were exposed to five acute concentrations (0.00ml/L, 0.025ml/L, 0.050ml/L, 0.075ml/L and 0.10ml/L) and the 96 h LC_{50} was 0.075 ml/L. The abnormal behavioural patterns noticed in the treated fish were erratic swimming, respiratory distress, discolorations of the skin, loss of reflex, hyperactivities, increasing opercula ventilation and excessive accumulation of mucus on the skin. There was an atretic follicle, cytoplasmic liquefaction, degeneration ovarian wall, invasion of granulosa layer and small inter-follicular spaces after treatment of 0.025 mg/L cypermethrin. Vacuolization at periphery, complete or partial rupture of maturing oocytes, vacuolization of oocytes, degenerating oocytes with disintegrated nuclei and alteration in structure were observed for 0.1 mg/L. The findings of this histoarchitecture analysis of ovary indicate a direct correlation between cypermethrin exposure and the histoarchitecture disorders.

Keywords: histoarchitecture; cypermethrin; *Heteropneustes fossilis;* LC_{50}; ovary

1. Introduction

Aquatic organisms, including fish, accumulate pollutants directly from contaminated water and indirectly via food chain (Sasaki *et al*., 1997). The aquatic environment is continuously being polluted with different toxic chemicals and pesticides those are used at culture ponds for treating parasitic fish diseases as well as applied to the agriculture lands are carried away by rains and floods as runoff to the water bodies (Richardson, 1988). Currently, there is no water body like pond, lake, river and sea environment that is entirely free from pollution, due to the consistent rise in the use of toxic chemicals. Pollution of water is any chemical, physical or biological changes in the quality of water that has a harmful effect on any living thing that drinks, uses or lives in (Lenntech, 1998). Exposure of organisms to xenobiotics such as pesticides, insecticides, herbicides and various kinds of chemicals is a serious matter in environmental and toxicological chemistry. Xenobiotics usually contaminate aquatic environment, a number of researchers have reported on the effects of different pesticides on aquatic organisms. Water quality parameters are influenced by the rate of pollutants entering the water or lethal effects on the aquatic organisms (Fagbenro, 2002; Olufayo, 2009). Aquatic environment polluted by pesticides that show some altered behavioral patterns which may include avoidance, locomotive activity and aggression, and these may be attempts by the fish to escape or adjust to the stress condition (Gormley and Teather, 2003; Morgan *et al*., 1991). As a result, the large mortality of aquatic life has been noticed due to direct or indirect attack of pollutants causing there is imbalance in their respiration, reproduction, excretion and osmoregulation etc. (Kharat *et al*., 2011).

Cypermethrin is a potent pyrethroids insecticide that generally used for the treatment of ectoparasites which infest cattle, sheep, poultry and some companion animals. Presently, it has been widely used as a chemotherapeutic agent for the control of ectoparasite infestations in marine cage culture and freshwater aquaculture (Hart *et al*., 1997; Boxaspen and Holm, 2001; Treasurer *et al*., 2004; Monir *et al*., 2015). These chemicals are potentially more toxic to fish and other aquatic organisms, and are least toxic to mammals. The excessive use of synthetic pyrethroids, the environment and water resources are being polluted, thus endangering aquatic life directly and human life indirectly (Hill, 1989). The half-lives for elimination of several pyrethroids by rainbow trout are all longer than 48 h, while elimination half-lives for birds and mammals range from 6 to 12 h (Bradbury *et al*., 1989).

Histoarchitecture effects of pesticides on various organs of aquatic animals were reported by many workers. Magar and Bias (2013) observed that histopathological impact in the ovary of the fresh water fish Taki, *Channa punctatus* exposed to malathion. Reddy *et al. (*1983) also studied that the sumithion affects on the ovarian growth of *Oziotelphusa sensex*. Victor (1984) observed histoarchitecture changes in ovary of freshwater prawn, C*aridina rajadhari* exposed to malathion. However, very few literatures are available on the impact of cypermethrin on histopathological aspects such as ovary in freshwater fish. Therefore, the present study reports histoarchitecture changes in the ovaries as a result of exposing Stinging catfish, Shing (*Heteropneustes fossilis*) is to sublethal concentration of synthetic pyrethroids, cypermethrin.

2. Materials and Methods

2.1. Collection of experimental fish

The experiment was conducted in the Fish Diseases and Health Management Laboratory, Bangladesh Fisheries Research Institute (BFRI), Mymensingh. Matured healthy female Stinging catfish, Shing (*H. fossilis*) were collected from cultured fish farm of Mymensingh area. The fishes were disinfected with 0.1% of potassium permagnate ($KMnO_4$) solution to avoid dermal infection. Fishes were then rinsed in water and acclimatized to the laboratory conditions up to 12 days in 75 L capacity of glass aquaria. During acclimatization fishes were fed with artificial pellet feed and water of the aquaria was also changed once in every day. Feeding was stopped 24 h prior to the commencement of the toxicity test experiment.

2.2. Experimental procedure

The cypermethrin toxicity test on the female *H. fossilis* was conducted in fifteen (15) glass aquaria and each aquarium contained 30 L of water where healthy female fish were introduced. For each experiment 10 fish of approximately similar size (20.32±1.4 cm in length) were exposed to 0.00ml/L (control), 0.025ml/L, 0.050ml/L, 0.075ml/L and 0.10ml/L concentration for 96 h of cypermethrin followed Monir *et al*., 2015. Each of the toxicant concentration was replicated three times. The LC_{50} of cypermethrin on the test fish was determined by using probit analysis. All data obtained in both tests were calculated using probit method and the graphical method (Finney, 1971).

2.3. Histoarchitecture examination of the ovary

After the 96 h experiment, tissue of ovary were dissected out from control and experimental *H. fossilis* and then the collected ovary were fixed in Bouins fluid separately. Preserved ovary were washed under tap water for 24 h to remove formalin, dehydrated, clarified with xylene and embedded in paraffin blocks. Then they were cut at 4-5µ thickness by using Elisa microtome and stained routinely with haematoxylin and eosin (H&E) for histoarchitecture examination. Stained histoarchitecture sections were examined under trinoculare microscope. Histoarchitecture changes of ovary were photographed and interpreted in comparison to the work of previous.

3. Results

3.1. Fish behaviour and mortality

During exposure, the treated *H. fossilis* showed abnormal behavioural such as erratic swimming, respiratory distress, discolorations of the skin, loss of reflex, hyperactivities, increasing opercula ventilation, vertical movement and excessive accumulation of mucus on the skin before death. The reaction to the cypermethrin was more pronounced in the aquaria containing the highest three (3) of 0.025 mg/l, 0.075 mg/l and 0.10 mg/l concentrations of the toxicant. The 96 h LC_{50} value was found 0.075 ml/L (Figure 1) but the lowest (12%) and highest (79%) mortality was observed in 0.025 and 0.10 ml/L cypermethrin respectively. However, the control group fish (not exposed to cypermethrin) showed normal behavior and no mortality were found during the experiment.

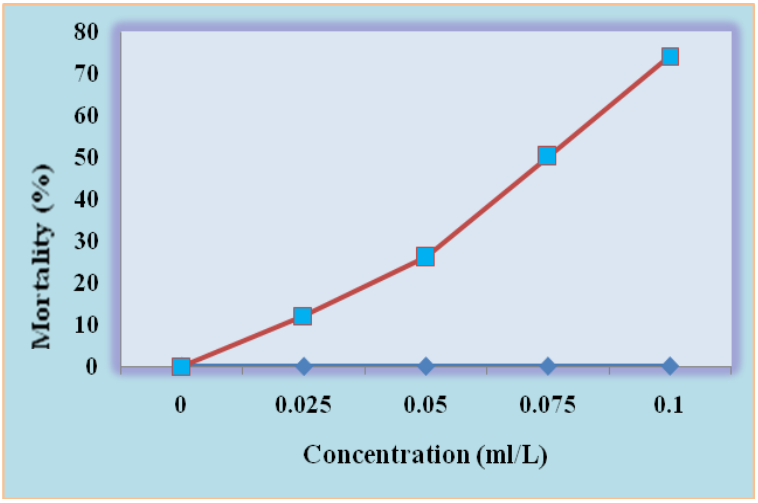

Figure 1. 96 h LC_{50} of freshwater Stinging catfish, Shing (*H. fossilis*) exposed to different concentrations of cypermethrin.

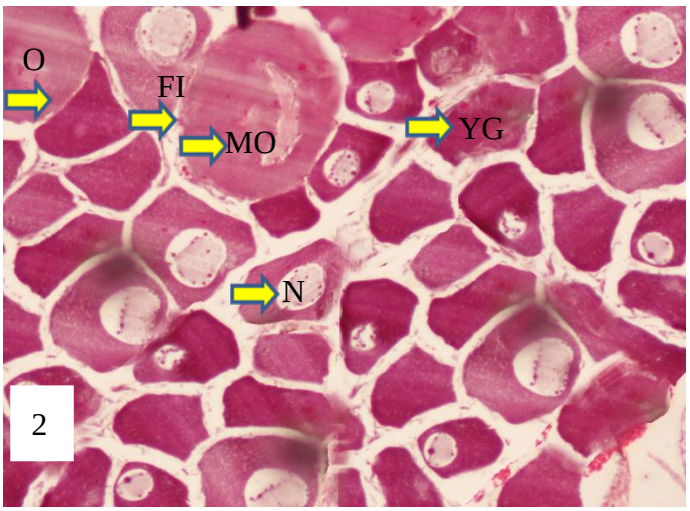

Figure 2. Ovary of *H. fossilis* in the control aquarium shows normal structure. Nucleus (N), Follicular lining (Fl), Mature oocytes (MO), Yolk granules (YG), Oocyte (O) H&E x 400.

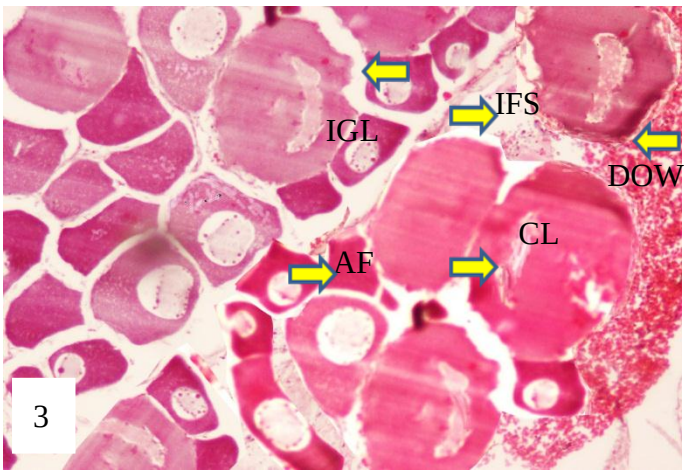

Figure 3. Ovary of *H. fossilis* exposed to 0.025 ml/L of cypermethrin shows Atretic follicle (AF), Cytoplasmic liquefaction (CL), Degeneration ovarian wall (DOW), Inter-follicular space (IFS), Invasion of granulosa layer (IGL) H&E x 400.

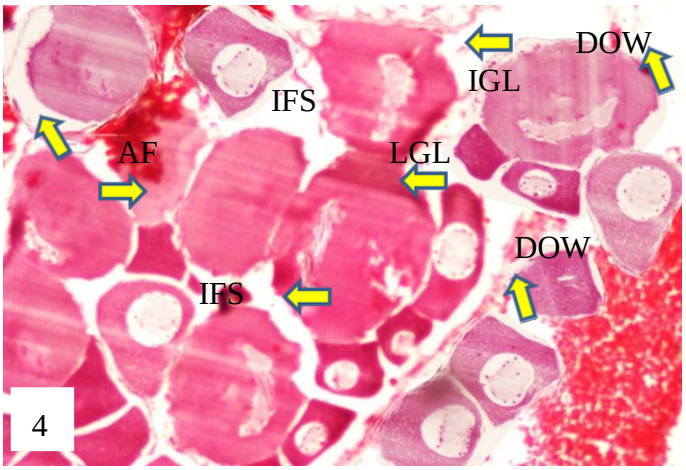

Figure 4. Ovary of *H. fossilis* exposed to 0.050 ml/L of cypermethrin shows Atretic follicle (AF), Cytoplasmic liquification (CL), Degenerated ovarian wall (DOW), Inter-follicular space (IFS), Invasion of granulosa layer (IGL), Lifting of granulose layer (LGL) H&E x 400.

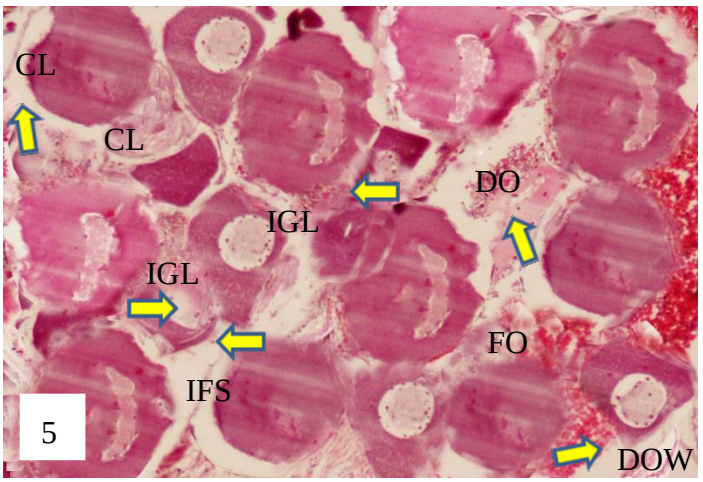

Figure 5. Ovary of *H. fossilis* exposed to 0.075 ml/L of cypermethrin shows Cytoplasmic liquification (CL), Degenerated ovarian wall (DOW), Degenerating ocytes (DO), Fragmented ova (FO), Inter-follicular space (IFS), Invasion of granulosa layer (IGL), H&E x 400.

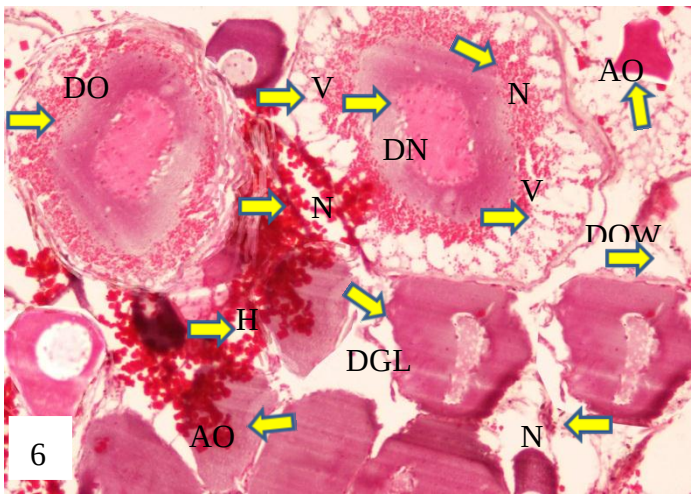

Figure 6. Ovary of *H. fossilis* exposed to 0.1 ml/L of cypermethrin shows Atretic oocyte (AO), Degenerated granulose layer (DGL), Degenerated ovarian wall (DOW), Degeneration of oocytes (DO) Destruction of Nucleus (DN), Hemorrhages (H), Necrosis (N), Vaculation (V) H&E x 400.

3.2. Effects of cypermethrin on the histoarchitecture of ovary

3.2.1. Control Ovary

The ovary of control *H. fossilis* were found large number of mature and maturing oocytes. The immature oocytes were transparent with nucleus and cytoplasm but the matured oocyte or vitellogenic oocytes were round opaque filled with small and large yolk globules (Figure 2).

3.2.2. Experimental Study

After exposure of different concentrations of cypermethrin as 0.025 ml/L, 0.050 ml/L, 0.075 ml/L and 0.10 ml/L for 96 h, the ovaries were found with different changes in their structural design. The changes of fish ovary that exposed to 0.025 ml/L of cypermethrin were found a few numbers of oocytes and small inter-follicular spaces. Atretic follicle, cytoplasmic liquefaction, degeneration ovarian wall and invasion of granulosa layer were also found (Figure 3). Cytoplasmic liquification, clumping of cytoplasm, ovarian wall rupture, displacement of nucleus and yolk vesicles and disappearance of nucleolus, invasion of granulosa layer were observed in some mature oocytes for exposure of 0.050 ml/L. (Figure 4). The degree of fish ovary histoarchitecture changes was related to the increasing concentration of cypermethrin. The observation of experimental fish that exposed to 0.075 ml/L of cypermethrin showed significant changes of the ovary. It was found destruction of epithelial layer, evidence of degeneration of oocyte, vacuolization at periphery, complete loss of normal configuration of ovary, disorganization of nucleus, elongated ovarian follicles with rupturing follicular epithelium and fragmented ova of altered shape (Figure 5). In higher concentration 0.10 ml/L of cypermethrin, there was complete or partial rupture of maturing oocytes, stromal hemorrhage, vacuolization of oocytes and general inflammation clumping of cytoplasm and degenerative oocytes became phagocytic and exhibited likely atresia. Necrosis and fibrosis in connective tissue and damage to yolk vesicles of maturing oocytes was also observed. Large inter-follicular spaces had been formed for the shrinkage of the oocytes and few oocytes were noticed in the process of absorption i.e. atretic oocytes (Figure 6).

4. Discussion

Generally, cypermethrin has been widely used as a chemotherapeutic agent for the control of ectoparasite infestations in marine cage culture and freshwater aquaculture (Hart *et al*., 1997; Boxaspen and Holm, 2001; Treasurer *et al*., 2004). The experiment revealed that the toxic effect of cypermethrin on the survival rate of the *H. fossilis* was assessed by the LC_{50} value calculated as 0.075ml/L at 96 h exposure and the abnormal behavior of respiratory impairment due to the toxic effect of cypermethrin was almost similar with the findings of Omitoyin *et al.* (2006) and Omoniyi *et al*. (2002) and Monir *et al.* (2015) that pesticide impairs respiratory organs. The abnornmal behavior such as erratic movement, loss of reflex, discoloration, changes in behavior and increased opercula ventilation, resting at the bottom of the treated fish due to the toxic effect of cypermethrin on the gills were similar to the observations of Aguigwo, 2002; Rahman *et al*., 2012.

The histoarchetecture abnormalities in ovaries may be caused by several conditions or factors such as ionizing radiations, electric current, parasitic infections, mechanical injuries, xenobiotic toxicants (Sarojini and Victor, 1985) and by a variety of effluents and aquatic pollutants (Shukla *et al*., 1984; Kumar *et al*., 2000). However, cypermethrin induced significant alteration in the ovary of the *H. fossilis*. There was a destruction of epithelial layer, inflammation clumping of cytoplasm, vacuolization at periphery, rupturing of follicular epithelium, vacuolization and degeneration of oocytes, and disorganization of nucleus after treatment of cypermethrin in this experiment. As increased in exposure leads to increase in abnormalities of the ovary. These changes of the treated ovary might be due to the direct effects of cypermethrin on developing oocytes for intervening the enzyme system in metabolism or destroying structure and function of hormone that control the ovarian growth (Kharat *et al*., 2011).

The findings of the present study were almost similar with that of Raju and Marutirao (2013), where they found degenerative oocytes, exhibited atresia and the maturing oocytes the granulosa layer gets separated and complete or partial rupture in the ovary of *Puntius ticto* after exposed of dimethoate. Pandey and Shukla, 1985; Singh and Sahai, 1995; Giri *et al.* (2000) conducted experiment on *Heteropneustes fossilis* by exposing insecticide basathrin and they observed massive damage in germinal epithelium, atresia of oocyte, stromal hemorrhage, vacuolization of oocytes and general inflammation. Srivastava *et al.* (2008) reported devicyprin induced many gonadal impairmante in a fresh water food fish *Channa punctatus* (Bloch). According to Kharat *et al.* (2011) the vacuolization at periphery, degenerating oocytes with disintegrated nuclei and vacuolization and alteration in shape was observed for of 0.09 ppm. exposed to TBTCl of Freshwater Prawn, *Macrobrachium Kistnensis*. The complete loss of normal configuration of ovary, necrosis, elongated ovarian follicles, and fragmented ova with abnormal shape were reported in *C. punctatus* exposed to melathione Magar and Bias

(2013). Hossain *et al*. (2002) in the ovaries of *Anabas testudineus* and *C. punctatus* after the exposure 0.5 and 5.0 ppm concentration of pesticide, dimecron 100SCW. Moreover, Giri *et al*. (2000) reported the effects of insecticide basathrin induced histoanatomical insult of ovarian tissue of catfish, *H. fossilis*. Rastogi and Kulshrestha (1990); Raju and Marutirao (2013) studied the effects of endosulfan, carbofuran and methyl Parathion on the ovary of carp minnow, *Rasbora daniconius* and they noticed atretic oocytes and increase in the interfollicular spaces. Almost same findings were observed by Kumar *et al*. (2000) in *C. fasciatus* under nickel toxicity. Benarji and Rajendranath, 1991; Raju and Marutirao (2013) studied cyto-architectural changes in the oocytes, including pronounced vacuolation, degeneration and deformation, clumping of the cytoplasm and karyohypertrophy in *Clarias batrachus* exposed to dichlorvos. These findings are strongly supported the present experiment in *H. fossilis*. Furthermore, Sakthival and Gaikwad (2001) found large number of atresia in the ovary of dimecron treated fish *Gambusia affinis.* Baruah and Das (2002) observed partial lysis, swelling, atresia and changes in nucleus and cytoplasmic organization after exposure of *Heteropneustes fossilis* to paper mill effluent**.** Raju and Marutirao (2013) noticed the effect of the diazinon on the ovaries of bluegill (*Lepomis micromeres*) that were cytoplasmic retraction in oocytes II, cytoplasmic degeneration, increased atretic oocytes, damages to the oocytes IV, partial destruction of the ovigarous lamellae and vitellogenic membrane, destruction of follicles and necrosis in the cytoplasm. However, almost similar results were reported by various authors (Shukla *et al*., 1984; Kharat *et al*., 2011).

5. Conclusions

The freshwater matured female Stinging catfish, Shing (*H. fossilis*) was exposed to various concentration of cypermethrin resulted that this synthetic pyrethroid was highly toxic to the *H. fossilis* with lethal concentration (LC_{50}) of 0.075ml/L. The present histpathological investigation demonstrates a direct correlation between cypermethrin exposure and considerable degree of histoarchitecture alteration in the ovary was noticed. Therefore, cypermethrin which is used in culture ponds especially in brood ponds as a treatment for parasitic diseases and disinfectant should be applied very carefully.

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Conflict of interest

None to declare.

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