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Histopathological Effects of Hexavalent Chromium in the Ovary of a Fresh Water Fish, *Channa punctatus* (Bloch)

Ashish Kumar Mishra · Banalata Mohanty

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Abstract The histopathological effects of hexavalent chromium (Cr VI) in the ovary of a fresh water teleost, *Channa punctatus* were investigated. An exposure-dependent alteration in ovarian histology is reported. For both acute and chronic exposures to Cr (VI), the percentages of atretic oocytes were increased; this increase was more pronounced in the acute exposure group. A decrease in percentage of vitellogenic oocytes was observed in the chronic exposure group indicating impairment of vitellogenesis. The hepatocellular vacuolization and atrophy along with pyknotic nuclei in both acute and chronic chromium exposed fish liver supports the vitellogenic impairment. The observed alterations may be due to both direct cytotoxic effect of Cr (VI) on the ovary as well as mediation by overall systemic toxicity affecting other vital organs.

Keywords Hexavalent chromium · Ovarian histopathology · *Channa punctatus*

Chromium (Cr), one of the important toxic heavy metals, is released to water bodies through the effluents of various industries. Its indiscriminate introduction into aquatic ecosystem may pose major threat to growth and survival of fish populations. Effects of chromium on the hematological (Gautam and Gupta 1989), biochemical (Jha and Jha 1995), and immune (Arunkumar et al. 2000) parameters as well as histological gill lesions (Nath et al. 1997; Begum et al. 2006) of fish have been reported. However, scientific evidence of toxicological impacts of chromium on the fish reproductive system is completely lacking. Recent studies in a few mammal species like mice (Pereira et al. 2005; Acharya et al. 2006), monkey (Aruldhas et al. 2005; Subramanian et al. 2006) and human (Li et al. 2001; Danadevi et al. 2003) determined that chromium acts as a reproductive toxicant. The focus of the present study was therefore, to investigate the Cr (VI) induced ovarian histopathology of a teleost fish, *Channa punctatus* both on acute and chronic exposures during the preparatory phase of the reproductive cycle. The vitellogenic growth (vitellogenin incorporation) of the ovarian follicles takes place during the preparatory phase. Since vitellogenin is synthesized in the liver and transported via blood to the ovary to be taken up by growing oocytes (Wallace 1985; Mommsen and Walsh 1988), the impact of chromium on the liver was also evaluated for the correlative assessment.

A. K. Mishra · B. Mohanty (✉)
Department of Zoology, University of Allahabad,
Allahabad 211002, India
e-mail: drbana_mohanty@rediffmail.com

Materials and Methods

Adult female specimens of the *Channa punctatus* (Order: Ophiocephaliformes, Family: Ophiocephalidae) weighing 50 ± 5 g, (length 18 ± 2 cm) were collected from clean and unpolluted local freshwater pond used for fish culture in March 2006, bath treated with 0.1% $KMnO_4$ solution and acclimatized to laboratory conditions for 2 weeks before experimentation. Fish were maintained in glass aquaria containing seasoned tap water (pH 7.3 ± 0.05, DO 7.5 ± 1.0 mg/L, total hardness 215.3 ± 7.0 mg/L as $CaCO_3$ and alkalinity 133.2 ± 5.0 mg/L as $CaCO_3$) under natural photoperiod (13L: 11D) and ambient temperature (18–21°C). Fish were provided with commercial dry fish feed pellets ad libitum (Hello fish dry pellets; CVM products, Beijing, China) at approximately 2%–3% of body weight of the fish/day.

The fish were divided into three groups of 12 individuals. Group I was the unexposed control, Group II and III were exposed to hexavalent chromium salt, potassium dichromate ($K_2Cr_2O_7$; MERCK, Mumbai, India). The LC_{50} value of $K_2Cr_2O_7$ was determined to be 41.75 mg/L using arithmetic method of Karber as adopted by Dede and Kaglo (2001). The chronic exposure to a sublethal concentration of 4 mg/L ($\approx$10% of 96 h LC_{50}) was applied for 1 month to Group II and the acute exposure of 20 mg/L ($\approx$50% of 96 h LC_{50}) was applied to Group III for 4 days. The acute exposure to Group III fish was begun on the 26th day of the experiment. The whole exposure medium was changed every other day in both the treatment groups to maintain the desired concentration of chromium salt. The water in control group was also changed at the same time. Fish were killed by decapitation; ovaries and liver were dissected out, ovaries were weighed, the tissues were then fixed in Bouin's solution for 24 h, and processed via standard histological procedures. Paraffin blocks were cut in transverse plane at 6-µm thickness and stained with haematoxylin and eosin for microscopic analysis.

Morphometric analyses of whole ovary as well as individual oocytes were carried out. The gonadosomatic index (GSI) was calculated in percentage as weight of the ovary (g)/weight of the fish (g) × 100. The diameter of individual oocyte stage was determined for 50 oocytes randomly selected from three tissue sections from each fish. For oocyte staging, 100 oocytes were randomly selected from each of the three different sections along the cranial–caudal axis of the ovary of each fish. The data obtained were expressed as means ± SEM and were analyzed with Student's *t*-test for statistical significance between experimental and control groups. The differences of the means were considered significant when $p < 0.05$.

Table 1 Effect of hexavalent chromium on the GSI and ovarian diameter in teleost fish, *Channa punctatus*

Parameters	Control	Acute	Chronic
GSI (%)	1.4 ± 0.12	1.28 ± 0.03	0.96 ± 0.04*
Ovarian diameter (mm)	2.4 ± 0.03	2.3 ± 0.04	2.1 ± 0.02**

* $p < 0.05$, ** $p < 0.001$, N = 10–12

Results and Discussion

In the control and chronically chromium (VI) exposed group no fish died while in the group exposed acutely to Cr (VI) two fish died. The death of fish in acute exposure group might be due to the systemic toxicity caused by high dose of chromium.

Fish exposed chronically for 1 month to 4 mg/L Cr (VI) showed retarded growth and development of the ovary as compared to control fish (Tables 1 and 2, Figs. 1a and b, 2a–c). The gonadosomatic index was significantly lower in comparison to control (Table 1). Unlike the uniform extension into the ovarian stroma in the control fish (Fig. 1a), the growth of the ovigerous lamellae were stunted in chronic exposed group (Fig. 2a). The diameters of all the three stages of oocytes of the preparatory phase ovary, previtellogenic non-yolky stage I oocytes and vitellogenic oocytes of stage II, III were reduced (Table 2) which resulted in a less compact arrangement (Fig. 2a). A lower percentage of vitellogenic oocytes were observed in the chronically exposed fish as compared to control fish (Table 2). Necrosis was observed in oocytes of all the three categories; previtellogenic stage I and vitellogenic stage II, III categories (Fig. 2a–c). The vitellogenic oocytes showed disrupted yolk platelets (Fig. 2b). Disruption of ovarian stroma was also pronounced (Fig. 2c). Increase in atretic oocytes was also evident in this group (Table 2).

Exposure for 96 h to 20 mg/L Cr (VI) did not alter the GSI or ovarian diameter as compared to control (Table 1). The percentage of previtellogenic oocytes of this group was also not different from the control group (Table 2). A significantly higher percentage of atretic oocytes were observed in ovaries from the acute Cr (VI) exposure group (Fig. 3a, Table 2). Atresia was mostly observed in the vitellogenic stage II and III oocytes (Fig. 3a and b).

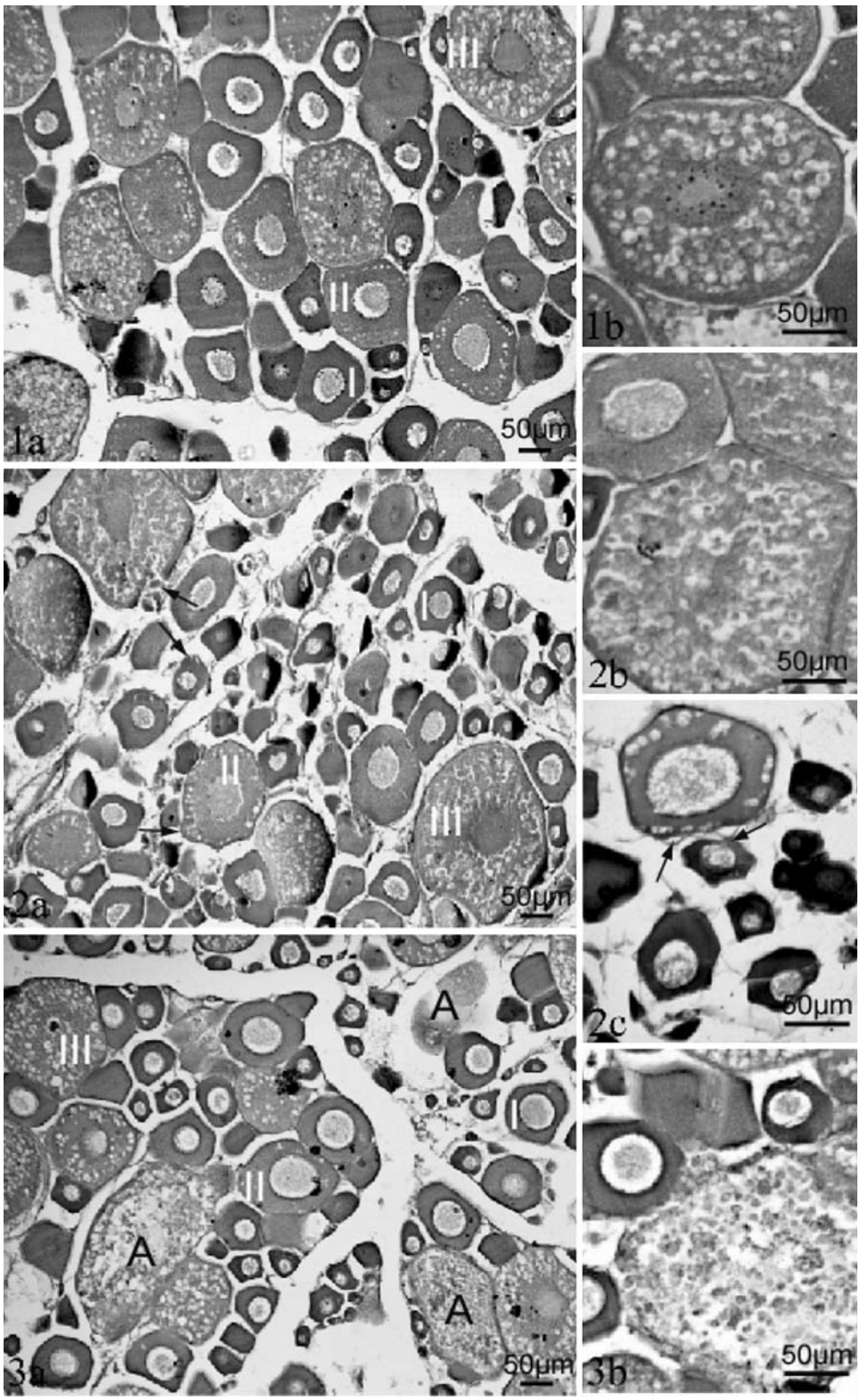
The necrosis of oocytes and ovarian stroma on chronic exposure to sublethal levels of Cr (VI) and increased

Table 2 Effect of hexavalent chromium on the number and size of Oocytes in teleost fish, *Channa punctatus*

Stages of oocytes	Control		Acute		Chronic	
	Number	Diameter	Number	Diameter	Number	Diameter
Stage I	57.5 ± 0.92	20–110	54.3 ± 1.3	20–110	76.0 ± 1.03**	15–75
Stage II	15.67 ± 0.49	120–160	15.0 ± 0.37	120–160	12.3 ± 0.6*	110–140
Stage III	25.3 ± 0.5	210–360	25.7 ± 1.4	210–360	9.3 ± 0.62**	180–360
Atretic	1.3 ± 0.2	160–275	4.5 ± 0.22*	160–275	2.0 ± 0.4	160–250

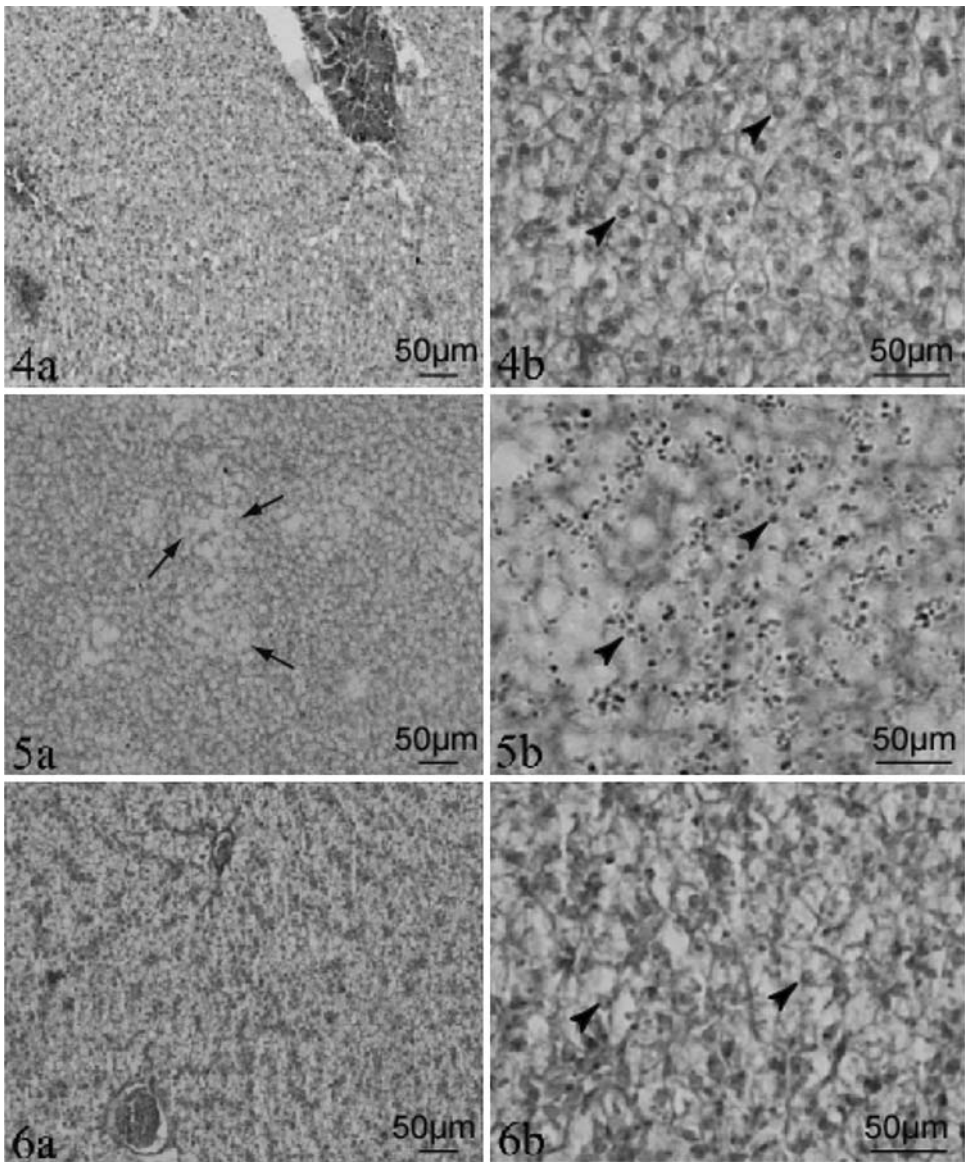
Numbers of oocytes are expressed in means ± SEM and diameters are in µm

* $p < 0.05$, ** $p < 0.001$, N = 10–12



Figs. 1–3 Transverse sections showing ovarian histopathology on Cr (VI) exposures. Fig. 1: Control. (**1a**) Note larger percentage of vitellogenic oocytes (80×); (**1b**) Magnified portion showing normal incorporation of yolk platelets in vitellogenic oocytes (200×). Fig. 2: Chronic exposure. (**2a**) Necrosis in oocytes of all the stages and larger percentage of previtellogenic oocytes with reduced diameter. (80×). (**2b**) Magnified view showing disruption of yolk platelets and (**2c**) Necrosis of vitellogenic and previtellogenic oocytes. (200×). Fig. 3: Acute exposure. (**3a**) Increased number of atretic oocytes (80×); (**3b**) Atretic oocytes in higher magnification (200×). I (previtellogenic), II, and III (vitellogenic) oocytes; A, atretic oocytes; Arrow, sites of necrosis

oocyte atresia in fish exposed acutely may all be due to the cytotoxic effect of the heavy metal. Chromium (VI) induced cellular toxicity in various organs of vertebrates has been reported (Aruldhas et al. 2005; Begum et al. 2006; Oliveira et al. 2006). Involvement of oxidative stress was suggested as one of the factors resulting in cellular toxicity (Aruldhas et al. 2005; Begum et al. 2006) since Cr (VI) on reduction generates intermediate species of Cr (V)



Figs. 4–6 Transverse sections showing histopathology of liver on Cr (VI) exposures. Fig. 4: Control. (**4a**) Regular parenchymatous architecture of hepatocytes (120×); (**4b**) Magnified view showing centrally placed nuclei in hepatocytes (480×). Fig. 5: Chronic exposure. (**5a**) Note sites of hepatocellular necrosis (120×); (**5b**) Magnified portion showing vacuolization as well as atrophy and disturbed nuclear arrangement with pyknotic nuclei in hepatocytes (480×). Fig. 6: Acute exposure. (**6a**) Prominent vacuolization in hepatocytes (120×); (**6b**) Vacuolated hepatocytes along with basal nuclei in higher magnification (480×); Arrow, sites of necrosis; (➤), nucleus

and Cr (IV) that further react with H_2O_2 to generate reactive oxygen species (ROS). It is likely that these ROS may interact with various tissues resulting in their damage.

The inhibition of vitellogenic growth of the oocytes was evident in the chronic Cr (VI) treatment group through the higher percentage of previtellogenic oocytes in comparison to the control group. The Cr (VI) mediated toxic effects may disrupt vitellogenesis by directly acting on the liver. Hepatic damage was well demonstrated histologically in both chronic and acute exposures as the normal histoarchitecture of the hepatocytes observed in the liver of control fish (Fig. 4a and b) was disrupted on chromium exposures (Figs. 5, 6). Localized degeneration of liver as well as hepatocellular vacuolization and atrophy were observed in the chronic exposure group (Fig. 5a and b). In comparison to central nuclear arrangement observed in the control, in the exposure group most of the nuclei were laterally placed and pyknotic (Fig. 5b). Hepatocellular vacuolization was more prominent in the acute exposure group (Fig. 6a, b), but less numbers of pyknotic nuclei were observed (Fig. 6b). The hepatocellular dystrophy with pyknotic nuclei in chronic exposure fish indicates cellular death; oxidative stress induced cellular apoptosis of Cr (VI) was reported (Blankenship et al. 1994). The vitellogenin synthesis in the teleost hepatocytes is also regulated by the hormones of the pituitary–ovarian axis (Dodd 1972; Ng and Idler 1983; Nagahama et al. 1993). It is thus probable that the inhibition of vitellogenic growth of oocytes in the present study may be due not only to the toxic impact of Cr (VI) directly on the ovary, but also to impacts on other components of the pituitary–ovarian axis that would result in the impaired synthesis and release of these hormones.

In conclusion, the present study elucidated the adverse effects of hexavalent chromium in the ovary of a teleost, *C. punctatus*; results also suggest that the overall toxic impacts occur at multiple organ sites. The lower percentage of vitellogenic oocytes in chronically exposed fish indicated that the long-term exposures to this heavy metal might pose a potential risk to fish populations in the vicinity of waters contaminated with hexavalent chromium.

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