

Research Article

Testicular Cycle of *Colisa fasciatus* (Bl. and Schn.) under Hexavalent Chromium Stress

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Abstract: Healthy specimens of *Colisa fasciatus* exposed to sub lethal concentration of hexavalent chromium (4.8 mg/L) for 15 days revealed no significant alterations in any testicular architecture during its different phases. However, 30 days exposure revealed distarded shape of lobule and lobular wall, scattered interstitial leydig cells during preparatory phase of testicular cycle where as during spawning phase of testicular cycle, dissolution of germinal epithelium, reduced intra lobular spaces and clumping of interstitial leydig cells was noticed. No apparent change was noticed during post spawning phase of testicular cycle. The findings indicate that hexavalent chromium interferes in the testicular physiology during its preparatory and spawning phases except post spawning phase of *Colisa fasciatus*.

Keywords: *Colisa fasciatus*, germinal epithelium, hexavalent chromium as potassium chromate, interstitial leydig's cell, intra lobular spaces, lobule and lobular wall, testicular cycle

INTRODUCTION

Some heavy metals including Lead, Nickel, Cadmium, Mercury etc., are toxic to living organisms even at low concentration, while others such as Copper, Iron, Zinc, Manganese etc., are biologically essential and natural constituent of the aquatic ecosystem and become toxic only at high concentration (Cohen *et al.*, 2001; Karadede-Akin and Ünlü, 2007; Storelli *et al.*, 2006). Hexavalent chromium is a well known highly toxic metal as compared to its trivalent form and considered as a priority pollutant. Industrial sources of Cr^(vi) include leather, tanning, plating, elctroplating etc., Cr^(vi) after discolution in aquatic environment is distributed in gills, skin and muscles of fishes (Yilmaz *et al.*, 2010).

Cr^(vi) is also used as a catalyst and coating material (Idachaba *et al.*, 2004). Grinding, welding and polishing of stainless steel are among principal ways of introducing chromium into the land environment while other ways of introducing chromium into aquatic environment include the waste incineration and the burning of fossil fuel (WHO, 1988). Pollution due to chromium may affect all ecosystem and human health directly or through food chain (Yilmaz *et al.*, 2009). Toxicity of chromium depends on its different oxidation states which have pronounced biological effects (Richard and Bourg, 1991). Further, it is to mention that Cr⁽ⁱⁱⁱ⁾ is less reactive than Cr^(vi) to a cell (Krenkel, 1974; Cohen *et al.*, 2001). In general, studies on toxicity using fishes have been confined to the determination of the lethal concentration in terms of

LC₅₀ values of hexavalent chromium (Adelman *et al.*, 1976) and its distribution in their tissues (Strokes and Framn, 1965; Yilmaz *et al.*, 2010) with little information available on the effect of sublethal doses of this metal on other parameters in general and gonadal cycle in particular. Chromium compounds have been reported to cause renal failure leading to the loss of osmoregulatory ability and respiration in fishes (Artillo and Meladio, 1988). However, reports pertaining to the deleterious effect of metallic pollutants especially chromium (vi) on the testicular cycle of teleost fishes is megre. Keeping this in view, present study has been undertaken to record the deleterious effect of sublethel concentration of hexaveleent chromium as potassium chromate, if any, on all three salient phases (preparatory spawning and post spawning) of testicular cycle of a freshwater perch, *Colisa fasciatus*.

MATERIALS AND METHODS

Adult male specimens of *Colisa fasciatus* (average length 6.4±1.6 cm and weight 7.2±1.04 gm) were procured from freshwater pond, acclimatized in dechlorinated tap water and treated with 0.1/KMnO₄ solution before being transferred into the experimental aquaria. They were fed powdered dried shrimp at alternate days. The properties of the tap water were as follows: temperature 21.2±1.4°C during preparatory phase and 25.4±1.6°C during Spawning and post spawning; pH 7.3; DO 6.8 ppm; hardness 122±2.4 mg/L as CaCO₃; electrical conductivity 1432±24.22 µho/cm. A sublethal concentration (4.8 ppm) of analytic grade of haxavalent chromium as potassium



Fig. 1: Showing normal structure of testicular lobule, lobular wall (→), Interstitial leyding's cell (→) during preparatory phase of testicular cycle of *Colisa fasciatus* HE X 1000

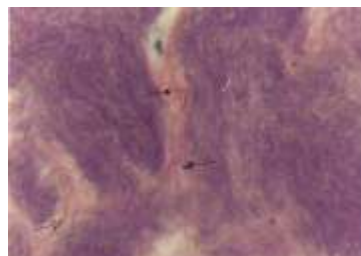


Fig. 3: Showing normal structure of lobule (→), lobular wall (→) and interstitial Leydig's cells (→) during spawning (late mature) phase of testicular cycle of *Colisa fasciatus* HE X 1000

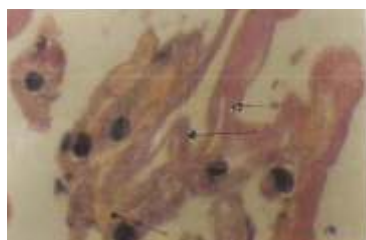


Fig. 2: Showing distarded shape of lobular wall (•→), distarded shape of lobule (→) and scattered Interstitial leydig's cells (→) during preparatory phase of testicular cycle of *Colisa fasciatus* under sub lethal concentration of hexavalent chromium stress for 30 days HE X 1000



Fig. 4: Showing dissolution of germinal epithelium (→), reduced Intra lobular spaces (→) and clumping of Interstitial leydig's cells (→) during spawning (late mature) phase of testicular cycle of *Colisa fasciatus* under sublethal concentration of hexavalent chromium stress for 30 days HE X 1000

chromate (K_2CrO_4) (Mark, India) was selected following the procedures outlined by Shukla (1988).

Fishes were divided into two groups (1st control and 2nd experimental); each having ten specimens in 25 L dechlorinated waters. 10 fishes were removed after 15 and 10 after 30 days from control and experimental tanks for histological observations during preparatory phase, late mature (spawning) and post spawning phase (1st week of March 09 to April 2009; 1st week June to 1st week of July 2010 and 1st week of Oct. 2010) respectively of the testicular cycle of *Colisa fasciatus*. The control was also run for 30 days.

Four days (24, 48, 72 and 96 h, repectively) static renewal bioassay toxicity test was performed to determine the LC_{50} values of hexavalent chromium as the methods outlined by APHA (1985). $1/10^{th}$ of LC_{50} for 96 h was selected as sub lethal concentration of hexavalent chromium for long term experimentation.

The testes of *Colisa fasciatus* from both groups were excised, washed and fixed in P.M.F. (Picro Mercurio Phormol). Paraffin sections (6-8 μ m) were stained with heidenhin's iron haematoxylin eosin. Student "t" test was applied for difference between experimental and respective control group with ($p < 0.05$) or less.

RESULTS AND DISCUSSION

A sublethal concentration of hexavalent chromium (4.8 mg/L) as Potassium chromate after 15 days of

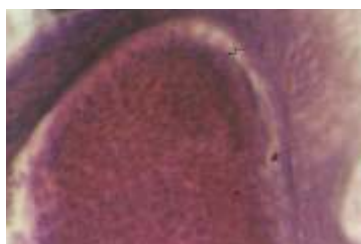


Fig. 5: Showing breaking and dissolution of germinal epithelium (→) necrosis and clumping of Interstitial leydig's cells (→), clumping of spermatozoa (→) during spawning (late mature) phase of testicular cycle of *Colisa fasciatus* under sublethal concentration of hexavalent chromium stress for 30 days HEX1000

exposure did not produce marked alterations in the architecture of the testis of *Colisa fasciatus* during preparatory as well as during spawning phase. However, 30 days of exposure in the same sublethal concentration resulted in noticeable structural and cellular changes in both the phases of testicular cycle i.e., preparatory and late mature (spawning phase) of *Colisa fasciatus*. Specimens during preparatory phase had misshapen and distorted shape of lobule and lobular wall and also scattered interstitial leydig, s cells as compared to control (Fig. 1 and 2). Their germinal epithelium showed breaking and dissolution at several

Table 1: Showing lobular diameter, thickness of the lobular wall, diameter of sperm mother cell, primary spermatocytes, secondary spermatocytes and interstitial leydig cells during preparatory phase and late mature (spawning phase) and early post spawning of testicular cycle under control and hexavalent stress

Phase	Lobular diameter	Lobular wall thickness	Sperm mother cell diameter	Primary spermatocyte diameter	Secondary spermatocyte diameter	Interstitial leydig cells diameters
Preparatory (control)	87.48±3.12	19.24±0.44	12.16±0.28	6.16±0.12	14.84±0.12	2.06±0.04
Preparatory (experimental)	80.24±2.22**	22.14±0.64	12.14±0.22	6.18±0.12	4.74±0.16*	1.78±0.06***
Late mature spawning (control)	140.28±3.02	48.22±3.16	12.22±0.16	6.14±0.10*	4.72±0.14	3.08±0.18
Late mature spawning (experimental)	130.61±2.52****	40.34±3.24****	12.24±0.18	6.26±0.14*	4.68±0.16*	2.44±0.16****
Early post spawning (control)	112.44±2.10	20.06±0.54	10.26±0.24	5.84±0.12	4.44±0.14	1.68±0.04
Early post spawning (experimental)	108.26±2.12*	19.12±0.42*	9.28±0.26*	5.24±0.16*	4.40±0.12	1.54±0.05*

*: Insignificant; **: p<0.05; ***: p<0.01; ****: p<0.001

places in addition to the intra lobular oedma that also occurs here and there, reduced intra lobular spaces, clumping of interstitial leydig's cells during spawning phase of *Colisa fasciatus* under hexavalent Chromium stress as compared to control (Fig. 3 and 4). Necrosis in spermatozoa & spermatids during spawning (late mature phase) was observed under stress (Fig. 5). Changes in the lobule diameter, labular wall thickness, sperm mother cell diameter, primary spermatocytes diameter, secondary spermatocytes diameter & interstitial leydig cells have been noticed as shown in Table 1 under sublethal concentration of hexavalent chromium stress as compared to control. However no apparent alteration in the testicular architecture under stress was noticed during post spawning phase of testicular cycle.

Numerous reports are available on the toxic effects of various metallic pollutants on fishes in general (Strokes and Framn, 1965; Pandey and Shukla, 1979; Pandey *et al.*, 1979; Pandey and Shukla, 1982, 1983; Shukla and Pandey, 1984a, b, c, d, e, 1985a, b, 1987a, b, 1988; Shukla *et al.*, 1985; Shukla, 1988; Znitkovich *et al.*, 1996; Storelli *et al.*, 2006; Karadede-Akin and Ünlü, 2007; Delemos *et al.*, 2001; Stemhagen *et al.*, 2004; Yilmaz *et al.*, 2009; Yilmaz *et al.*, 2010). However, toxic effect of hexavalent chromium, if any, one the reproductive cycling (testicular and ovarian) is scarce, Present study has, therefore, been undertaken to assess to deleterious effects of sub lethal concentration of hexavalent chromium as potassium chromate on the testicular architecture and steroid secreting cellular sites in a teleost fish, *Colisa fasciatus* during preparatory and late mature (spawning) phase of testicular cycle. Degeneration of spermatocytes and spermatids and lobular oedema has been attributed to manganese intoxication in rat testis (Chandra, 1971; Seth *et al.*, 1973). Following lindane treatment in the rat, massive, degenerative changes in the seminiferous epithelium and the intra and interlobular region has been noticed. Breaking and dissolution of the lobular wall in the preparatory and spawning phase of the testicular cycle, indicates a degenerative impact of hexavalent chromium. Our observation pertaining to the impact of

hexavalent chromium on the three phase of testicular cycle of *Colisa fasciatus*, are almost similar to those resulting from direct toxicant administration to mammals (Diksith and Datta, 1972). In fish, *Colisa fasciatus*, Cr^(vi) additionally produced statistically significant reduction in the diameter of Some cells appear to have undergone necrosis and cytolysis. These changes, in the two phases (preparatory and late mature (spawning) of testicular architecture appear to be duration dependent as 15 days exposure to sublethal concentration produced no such changes. Degenrative changes in the testis may be associated with decreased nucleic acid metabolism as evident from reduced diameter of interstitial leydig cells which are steroidogenic in nature. From our findings, it may be inferred that sublethal concentration of Cr^(vi) may hamper the testicular architecture and steroidogenesis of testicular cycle causing impairment in the normal reproductive physiology of the male fishes.

Causes for a little apparent change in the early post spawning phase of testicular cycle may be attributed to exhaustion of sperms and thus reveals static function.

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