Histopathological Analysis of Chromium Toxicity to Testis of the Catfish Clarias batrachus (Linn.)

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Abstract: *The present investigation has been conducted to study the sublethal toxic effect of chromium 14.2 ppm (40% of 96h LC_{50}) on the testis of the fresh water fish Clarias batrachus for a period of 45days. The histopathological changes observed as distortion of seminiferous tubules, disorganization of spermatogonia, spermatocytes and spermatids with cytoplasmic vacuolization and nuclear pycnosis. These alterations in the histology of testis in the fish Clarias batrachus exposed to sublethal chromium solution may be due to toxicity induced by the irritant present in the aquatic medium. Hence these changes can be used to assess the quality of water contaminated with heavy metal salt(s).*
Key words: *Histopathology, testis, chromium toxicity, Clarias batrachus.*

I. Introduction

Heavy metal concentration has been increased recently as a result of domestic, industrial as well as agricultural activities and poses greatest threat to the ecosystem [1], thus causing adverse effect on the fish and other aquatic organisms. Among heavy metals, Chromium is being used in organic chemicals, electroplating, iron, steel, electrical, paint, pigment manufacturing and leather tanning industries [2-4]. The effluents from these processes are strongly acidic and may contain the toxic hexavalent chromium and it is also derived from the oxidation of ores, combustion of fossil fuels, wood and paper [5]. Although eco-toxicological manifestations of chromium intoxication have been documented by several workers in various organs of fishes [6-11], the information regarding the adverse effect of sub-lethal chromium on the reproductive system of the fish C. batrachus are scanty as healthy gonads of fish are an important determinant of its breeding potential, and thus any toxicological factor adversely affecting the histopathology of gonads will definitely reduce the gross production of fishes. Hence, the present study was undertaken to assess the eco-toxicological manifestation of sub-lethal Chromium on testis of the freshwater fish Clarias batrachus.

II. Materials And Methods

2.1 Collection and maintenance of fish: Irrespective of sex, healthy specimens of male C. batrachus (length 10 ± 0.5 cm and 12 ±1 g of body weight) belonging to a single population were collected locally and were acclimated to the laboratory condition for 30 days (d). Water was renewed after every 24 hours (h) with routine cleaning of the aquaria. Fish were fed with minced goat liver every day.

2.2 Calculation of LC_{50}: Prior to the commencement of the experiment, 96 h median lethal concentration (96 h LC_{50}) of potassium dichromate (99% pure, E – Merck, India) was estimated following trimmed spearman karber method [12] and 24 h renewal bioassay system and was found to be 35.50 ppm after 5% trimming.

2.3 Experimental protocol: For the present study 14.2 ppm (40% of 96h LC_{50}) was selected as sublethal concentration as chromium present in natural water is up to 13 ppm [4]. Four groups of 10 fish each were exposed to 14.2 ppm potassium dichromate solution prepared in tap water having dissolved oxygen 6 ppm, pH 7.5, water hardness 40.44 mg L^{-1} and water temperature 28 ± 2°C [13]. Parallel group of 10 fish were kept in separate aquaria containing 50L of tap water without the addition of potassium dichromate as control. Feeding was allowed in the experimental as well as control groups' every day for a period of 3h before the renewal of the media throughout the tenure of the experiment.

After the expiry of 5, 10, 30 and 45 d of exposure, five fish each from the respective experimental as well as control groups were sacrificed, the testis were removed, preserved in 10% formalin for 24 hours at room temperature. Tissues were repeatedly washed with 70% alcohol till all the traces of fixative were removed. Dehydration process was carried out by washing the tissue with alcohol (90% and 100%), this is followed by cleaning process by alcohol benzene in different ratios(3:1, 1:1 and 1:3) followed by pure benzene and benzene-paraffin wax (1:1) then embedded in paraffin wax. 5μm sections were stained with haematoxylin and eosin. Permanent slides were prepared with Canada balsm and observed under light microscope.

The density (number) of spermatocytes was calculated following standard statistical procedures based on random sampling of five different sites from three control as well as experimental fish of each sacrificing

interval. One way analysis of variance (ANOVA) followed by Duncan's multiple range test was performed to determine whether spermatocyte density was significantly affected by exposure periods.

III.   Results

3.1 Control testis: Testes of C. batrachus are paired organs found in the abdominal region and each is enclosed in a peripheral connective tissue sheath. The innermost layer of this sheath, tunica propria, projects into the lumen of testis forming the seminiferous tubules. These tubules are lined internally with tubular or seminiferous or spermatogonic epithelium which gives rise to spermatocytes. The spermatocytes are later transformed into next developmental stage of spermatids and then to spermatozoa. Masses of spermatozoa can be seen lodged in seminiferous lobules, located at the blind ends of seminiferous tubules. This lobular part can be distinguished into somatic cells and germ cells (Fig 1). The central portion of the testis is made up of glandular tissue consisting of large and spherical interstitial glandular cells, fibroblasts, blood and lymph vessels. Sertoli cells were normal with granular cytoplasm containing large nuclei with a peripheral nucleolus. In a number of places inter lobular septa were marked by the presence of small and large aggregations of polygonal leydig cells. They have large nuclei with granualar chromatin and two or more nucleoli (Fig.1). The number of clearly visible primary spermatocytes is 397.60 /mm^2/seminiferous tubule in the control testis. The secondary spermatocytes are 681.50 mm^2/ seminiferous tubule (Table 1).

3.2 Experimental testis: After 5d of exposure, the testis showed inflammatory response. Slight disruption in seminiferous tubules was noted (Fig 2). The number of primary and secondary spermatocytes were decreased significantly ($p > 0.05$) as 340.8/mm^2 and 681.50/mm^2 respectively from their respective control groups (Table 1). After 10d of exposure, vacuolization, fluid filled seminiferous tubules and immature spermatogonia and general inflammatory response is observed (Fig. 3). At this stage the number of clearly visible primary and secondary spermatocytes are less (284.0/mm^2 and 553.80/mm^2) when compared with their control groups (Table 1). 30d of exposure is characterised by significant decrease in the primary spermatocyte (227.20/mm^2) as well as secondary spermatocyte (454.40mm^2) number and condensation of spermatogonic cells with inflammation, contraction and vacuolisation of tubules (Fig 4 and table 1). 45d of exposure is characterised by the disorganization of spermatogonia and degeneration of spermatids (Fig 5) and the interstitial component contain small cells, less cytoplasm (Fig 6). Due to the clubbing of the tubules the number of primary spermatocytes was decreased significantly as 198.8/mm^2 and secondary spermatocytes 340.80/mm^2after 45d of exposure ($p < 0.05$; Table 1). Extensive cytotoxic damage, general inflammatory response and other histological abnormalities are quite prominent. Although, inter-tubular vacuoles are clearly visible in all the experimental groups, the extent of histological damage, as is evident by the presence of large number of both inter and intra-tubular vacuoles, was maximum after 45d exposure period. The extent of vacuolisation in tubular epithelium increases with the increase in the duration of the experiment. Inflammatory cells are seen in the testicular tissue of every treated fish. In addition to gross vacuolisation and inflammatory response, distortion of seminiferous epithelium is quite prominent (Fig 6).

Table 1. Showing the variations in density (/mm^2) of primary and secondary spermatocytes in the testis of the fish Clarias batrachus for 5, 10, 20 and 45 days of sublethal chromium exposure.

Cells	Control	Experimental			
		5d	**10d**	**30d**	**45d**
Primary spermatocyte	397.60 ± 21.50*	340.80 ± 32.42*	284.00 ± 18.75*	227.20 ± 22.16*	198.80 ± 20.10*
Secondary spermatocyte	681.50 ± 16.18*	624.80 ± 26.12*	553.80 ± 19.15*	454.40 ± 24.50*	340.80 ± 21.75*

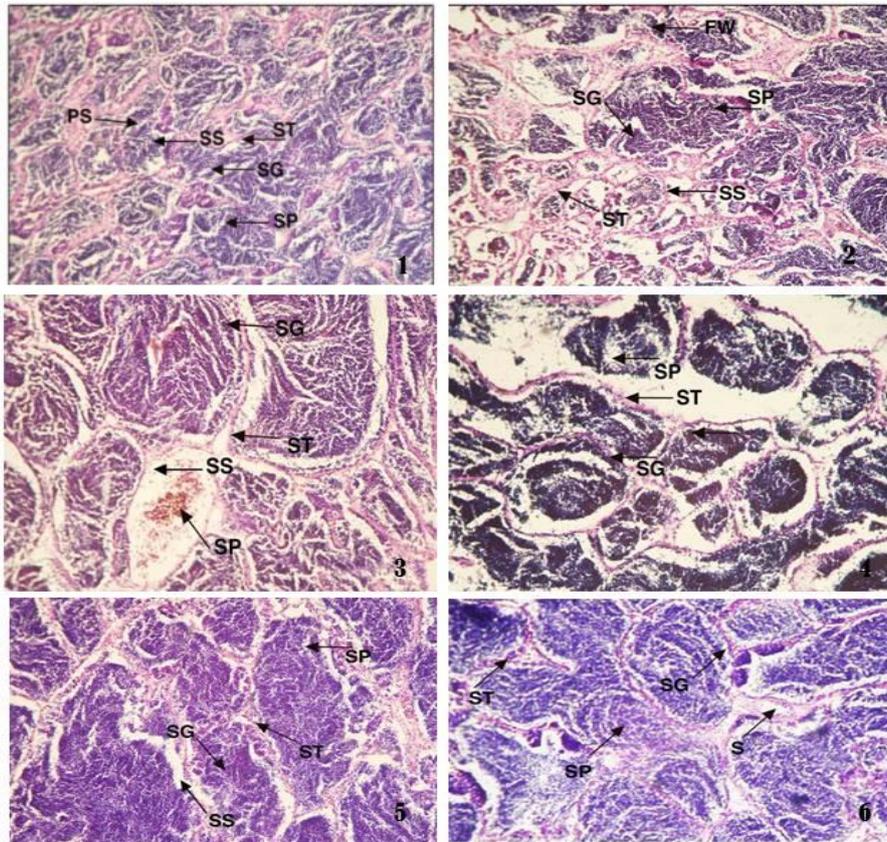*Average Values ± SE; n=3; *$p > 0.05$

Fig 1. Transverse Section (T. S) of testis of control fish showing normal architecture (PS:Primary spermatocytes, SS: Secondary spermatocytes, ST: Spermatids, SG: Spermatogonia, SP: Sperms) (H/E x 200). **Fig. 2** T. S of testis showing inflammation and slight disruption in seminiferous tubules after 5d of exposure (H/E x 200). **Fig. 3** T. S of testis showing vacuolization, fluid filled spaces and immature spermatogonia after 10d of exposure (H/E x 200). **Fig 4.** T. S of testis Showing condensation of spermatogonic cells, contraction and vacuolisation of tubules after 30 d of exposure (H/E x 200). **Fig 5.** T. S of testis Showing the disorganization of spermatogonia and degeneration of spermatids 45d of exposure (H/E x 200). **Fig 6.** T. S of testis Showing the interstitial component contain small cells with less cytoplasm (H/E x 200).

IV. Discussion

Fish exposed to sub lethal concentration of chromium at different exposure periods showed considerable degree of alteration in the histology of testes. In testes the seminiferous tubules are normally of varying shapes and sizes, each tubule has a definite thin fibrous wall which is not distinguished after spawning. The testes of C. batrachus showed highly conspicuous changes to sub lethal concentration of chromium in the form of reduction in the number and condensation of spermatogonic cells as well as inflammation of cells ,contraction and vacuolisation of tubules. Testicular inflammation was documented as one of the common responses in both aquatic and terrestrial animals exposed to environmental toxicants [14,15]. Zutshi [16] observed the effect of fenthion on the testes of Glossogobius giuris and observed reduction in size with spermatids and sperms in degenerating condition. In the present study, extensive destruction of the germinal elements in the testis of Clarias batrachus was observed after sublethal exposure to chromium. The mature stages underwent extensive atrophy. The histological changes observed in the testis of the fish might be caused by the disruption of the blood–testis barrier with a consequent metal accumulation in tissue [17]. Changes in the permeability of the blood-testis barrier [18] and alterations in testicular and epidydimal histoarchitecture [19] were also demonstrated in mice exposed to chromium. Further, Ram and Sathyanesan [20] and Crump and Trudeau [21] observed the inhibition of spermatogenesis in fish and testicular impairment is attributed to the direct cytotoxic effects of heavy metal as well as disruption of endocrine function. The results of the present study indicate that the presence of chromium in water can alter the histology of testis in fish Clarias batrachus.

Acknowledgments

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