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Research Paper

CADMIUM INDUCED HISTOPATHOLOGICAL CHANGES IN THE TESTIS OF THE FRESH WATER FISH, *Rasbora dandia*

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Abstract

The aquatic ecosystems are severely affected from global pollution because the final destination of pollutants released elsewhere on the earth is hydrosphere. Xenobiotic chemicals interfere with the normal physiological processes of aquatic organisms leading to early mortality and sterility. In the present study, reproductively mature freshwater fish, *Rasbora dandia* were exposed to sublethal concentration of 1 ppm of cadmium for 30 days period. Histopathological changes in the testis induced by the cadmium are studied. Gonadosomatic index was found to be progressively declined as the exposure period of cadmium increases. Blood vessels appeared to be dilated and collapsed by 30 days of cadmium exposure. Other histopathological changes like reduced spermatozoa and detachment of basement membrane are also appeared. The present study implies that chronic cadmium exposure induces testis damage in fishes leading to impaired reproductive success.

Key words: Histopathology, cadmium, testis, gonadosomatic index, *Rasbora dandia*.

INTRODUCTION

Over the last few decades, pollution is increasing at tremendous rate. The aquatic ecosystems are severely affected from global pollution because, the final destination of pollutants released elsewhere on the earth is hydrosphere. Often chemicals are disposed into water bodies without sufficient toxicological testing all over the world. Xenobiotic chemicals interfere with the normal physiological processes of aquatic organisms leading to early mortality and sterility. Wild fish populations are ever declining and many fish species are on the verge of extinction. Developing strategies for identifying chemicals causing damage to organisms is a necessity in the modern era.

Among the pollutants, heavy metals are highly toxic, persistent and affect the human and wildlife. Cadmium is a ubiquitous heavy metal which poses great potential hazard to humans and the environment as its uses are rising [1]. Cadmium is a readily bioaccumulating toxic metal in aquatic organisms and it occur in nearly all tissues. Cadmium has binding affinity similarities with nutritionally required elements like zinc, copper and calcium, resulting in cadmium protein interactions [2]. Normal physiology and homeostasis of organisms are often disrupted by non essential metals like cadmium in polluted ecosystems. Exposure of fishes to heavy metals not only disrupts reproductive hormone secretion, but also induces some pathological changes [3]. Conclusive studies on reproductive dysfunction of major xenobiotic

chemicals are inevitable. Apart from identifying harmful chemicals, strategic environment monitoring programmes can also be developed from these studies. The present study is our attempt to evaluate the histopathological changes in the testis of *Rasbora dandia* induced by cadmium.

MATERIALS AND METHODS

Rasbora dandia were collected from freshwater bodies of Thrissur, Kerala. These fishes were transported to the laboratory carefully and acclimatized to the laboratory conditions. Fishes with almost 5.5 to 7 cm length and 1.5 – 4 gm weight were selected and 10 fishes were kept in each aquarium. Cadmium chloride was used as source of the cadmium. To calculate lethal concentration, fishes were exposed to 10, 12, 14, 16 and 18 ppm of cadmium. Number of mortality in each aquarium is recorded on every 12 hours for a period of 96 hours. From this reading, LC_{50} value was found out using probit analysis. 96 hours LC_{50} concentration of cadmium was found to be 16.91 ppm of cadmium.

Experimental fishes were exposed to sublethal concentration of 1 ppm of cadmium for 30 days. Test solutions are renewed every 24 hours and fed with standard fish feed. Fishes were anaesthetised and scarified on every 10 days time interval during the experiment. Testis were dissected out and placed in 10% formalin for fixation. Testis were dehydrated in ethanol and cleared in xylene. Specimens were then infiltrated with paraffin wax and embedded in paraffin blocks. 1-3 µm sections were taken using rotary microtome and stained using haematoxylin and eosin. Histopathological changes were observed under light microscope. Gonadosomatic index (GSI) was calculated using the method followed by Kirubagaran and Joy [4]. Student's t-test was used for statistical analysis of GSI.

RESULTS

Gonadosomatic index was found to be progressively declined as the exposure period of cadmium increases (Table 1). Control testis of *Rasbora dandia* appeared to be filled with abundant spermatozoa (Figure 1). By 10 days of cadmium exposure, ripe spermatozoa appeared to be declined (Figure 2). Vacant spaces increased inside lobules due to the disappearance of spermatozoa (Figure 3). Some areas showed disorganised lobules in the testis of 20 days cadmium treated fishes (Figure 3). Blood vessels appeared to be dilated and collapsed by 30 days of exposure. This resulted in the invasion of nucleated ellipsoid red blood cells into lobules (Figure 4). Detachment of basement membrane occurred in the testis of cadmium exposed fishes (Figure 5).

DISCUSSION

Cadmium is a widespread non essential metal raising environmental concern [2]. According to Jarup, (2003) [5], instead of recycling, cadmium containing products are often dumped with household waste which attributed the dramatic increase of cadmium emissions during the 20th century. It is established that cadmium can affect the reproduction of fish populations through gonadal bioaccumulation and endocrine disruption, but few studies investigated non lethal interaction of cadmium in fish [6].

GSI of test fishes are found to be gradually decreased by cadmium exposure in the present study. Vergilio *et al*., (2015) [7] observed reduced GSI along with increased morphological alterations in the tropical fish *Gymnotus carapo* exposed to cadmium concentrations higher than 10 µM. According to Kime, (1999) [8], GSI is the simplest method for understanding gonadal dysfunction as reduced GSI indicates decreased activity of reproductive axis.

Mature spermatozoa were less abundant in test fishes compared to controls. Gamete development is disrupted by heavy metals which induce metallothionein synthesis, disturbing normal zinc homeostasis [8]. By 30 days of cadmium exposure in *Rasbora dandia*, blood vessels appear damaged resulting in the invasion of red blood cells into lobules. Kumari and Dutt [9] observed disrupted vascularization and hemorrhagic necrosis in *Punctius sarana* exposed to cadmium chloride. Sangalang and O'Halloran [10] reported that by cadmium exposure some

blood vessels of testis of brook trout collapsed and large number of red blood cells were found surrounding the lobules. The results of the present study imply that chronic cadmium exposure induces testis damage in fishes. Reduced GSI and less abundant mature sperms indicate impaired reproductive success.

Zhou *et al*., [11] suggests that biomonitoring is an appealing tool for the assessment of ubiquitous metal pollution in aquatic ecosystem. According to Hutchinson *et al*., [12] secondary sexual characteristics, gonadosomatic indices, plasma steroids, and gonadal histology are significant as biomarkers for guiding interspecies assessments of endocrine disrupting chemicals and designing fish chronic tests. Toxicological data on the effect of major aquatic pollutants on different fish species provide sufficient comparative data, facilitating the development of suitable biomarkers.

Table 1. Effect of cadmium exposure on GSI of male *Rasbora dandia* (Mean ± SD)

Exposure period	Control fishes	Fishes exposed to 1 ppm cadmium
10 days	6.08 ± 0.29	4.42 ± 0.48*
20 days	6.57 ± 0.59	2.44 ± 0.25*
30 days	6.04 ± 0.64	1.66 ± 0.28*

* Significant at $p < 0.01$

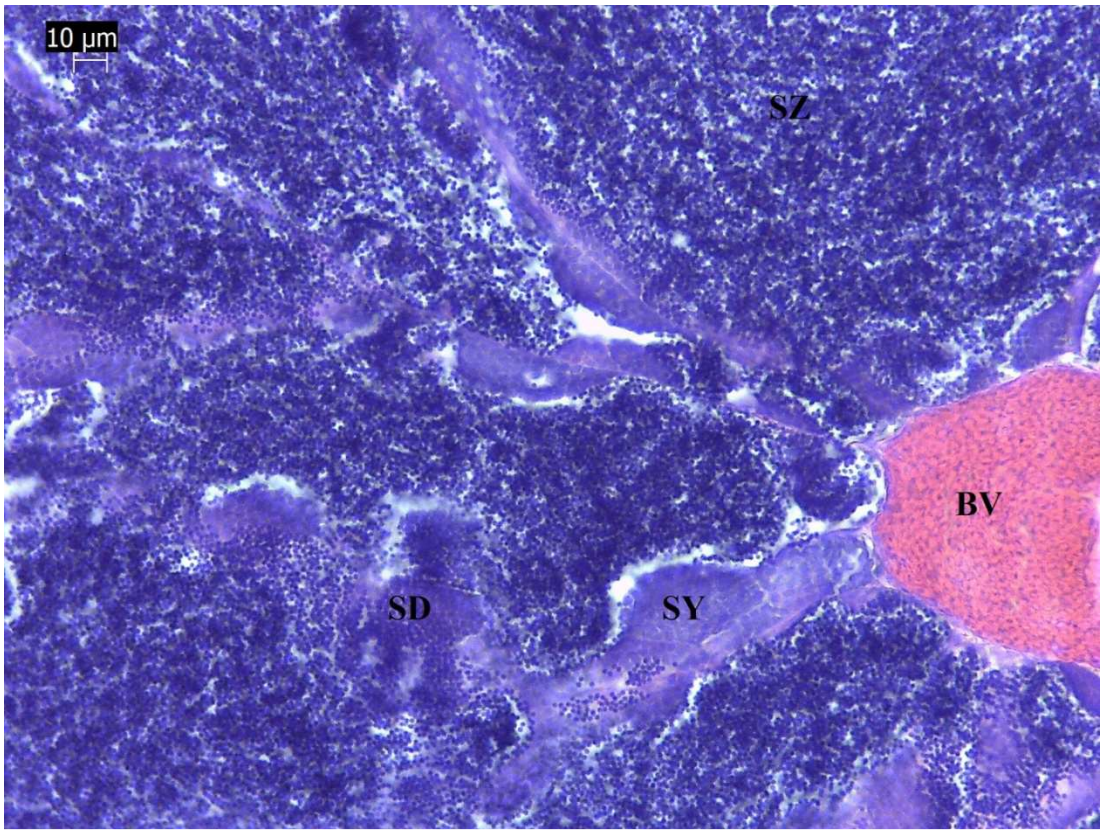

Fig 1: Histological section of testis of *Rasbora dandia.* (control); BV- Blood vessel, SZ- Spermatazoa, SY- Spermatocytes, SD- Spermatids

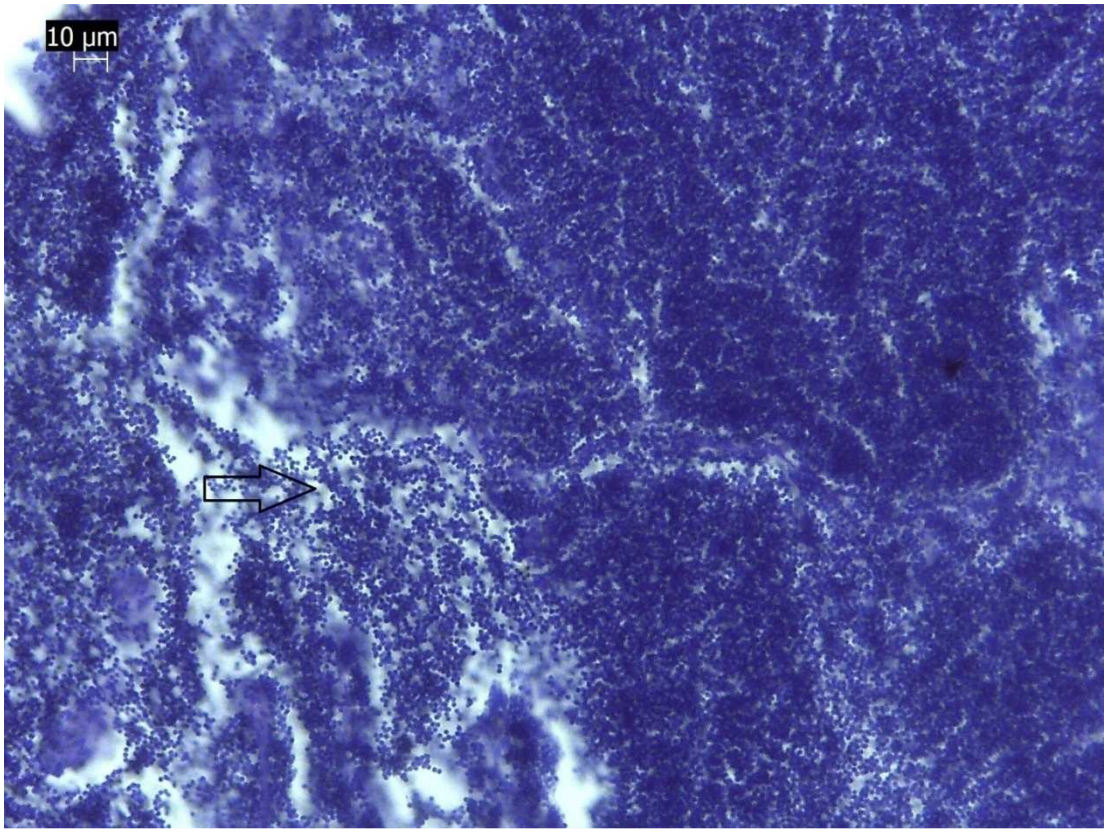

Fig 2: Histological section of 10 days cadmium exposed testis of *Rasbora dandia*; ⇨ Ripe spermatozoa declined ( )

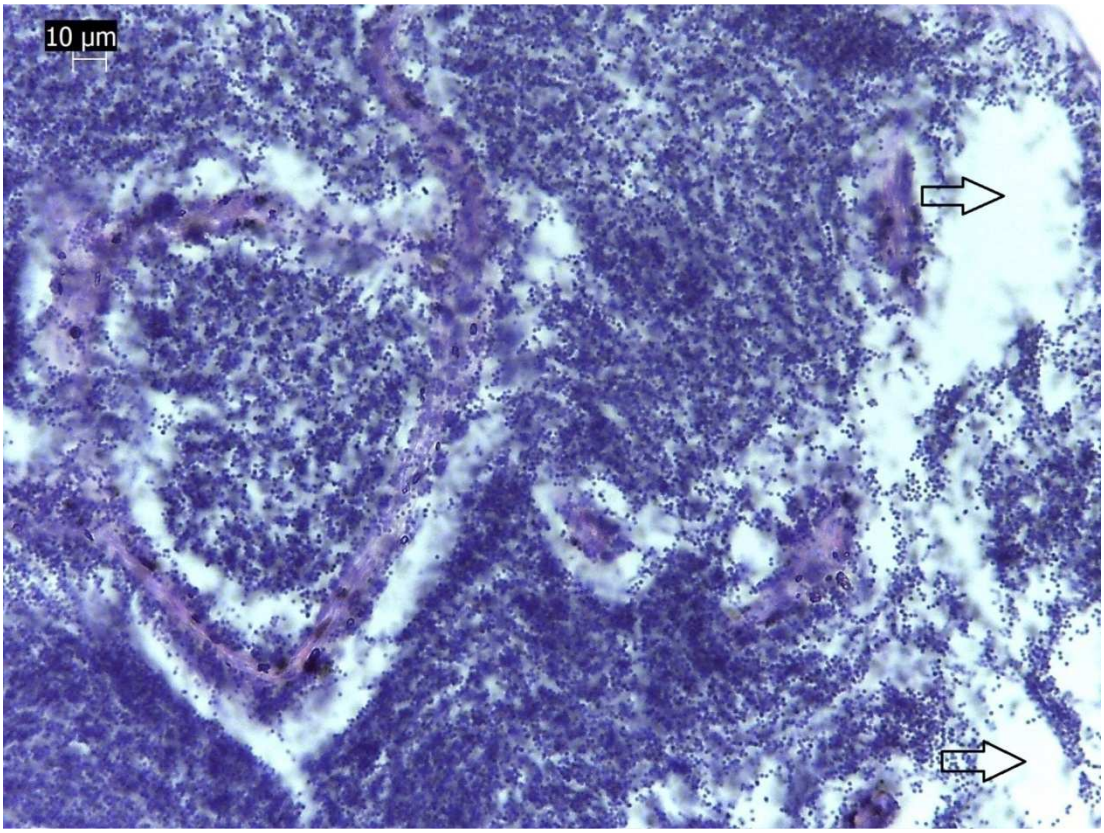

Fig 3: Histological section of 20 days cadmium exposed testis of *Rasbora dandia*; vacant spaces increased by reduction of spermatozoa (⇨)

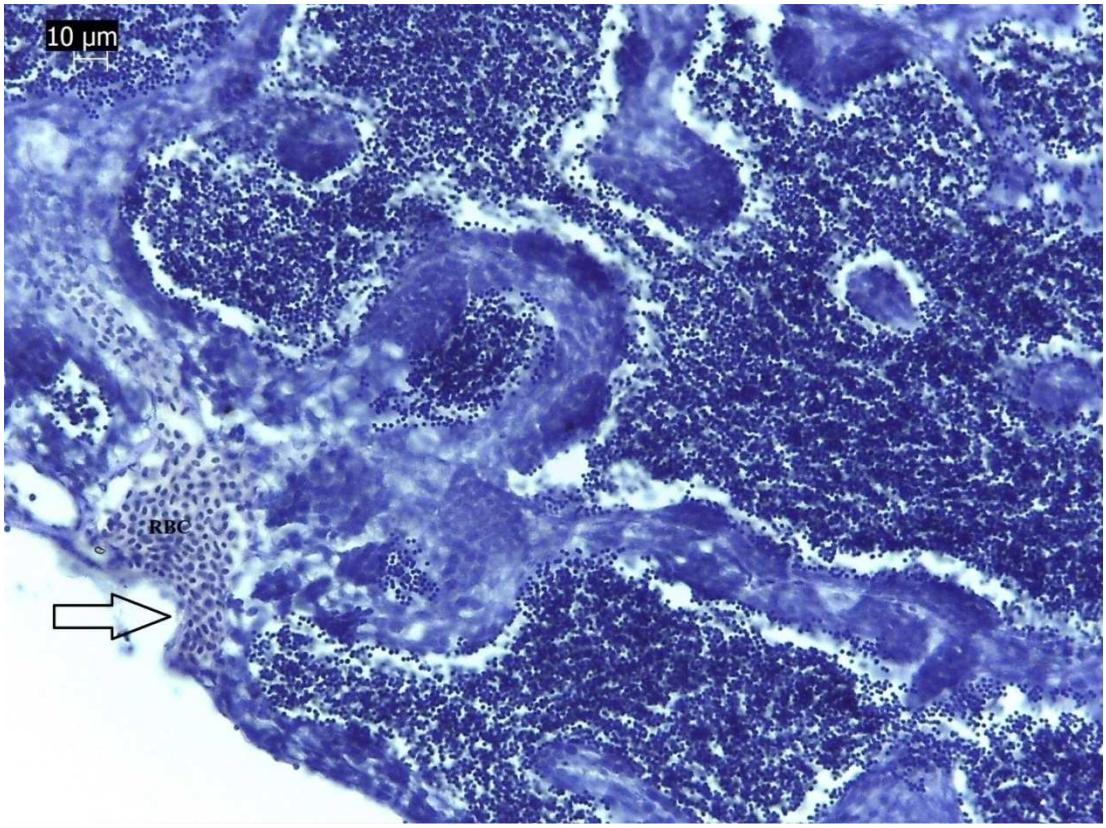

Fig 4: Histological section of 30 days cadmium exposed testis of *Rasbora dandia*; Dilated blood vessel (⇨), RBC- Red blood corpuscles

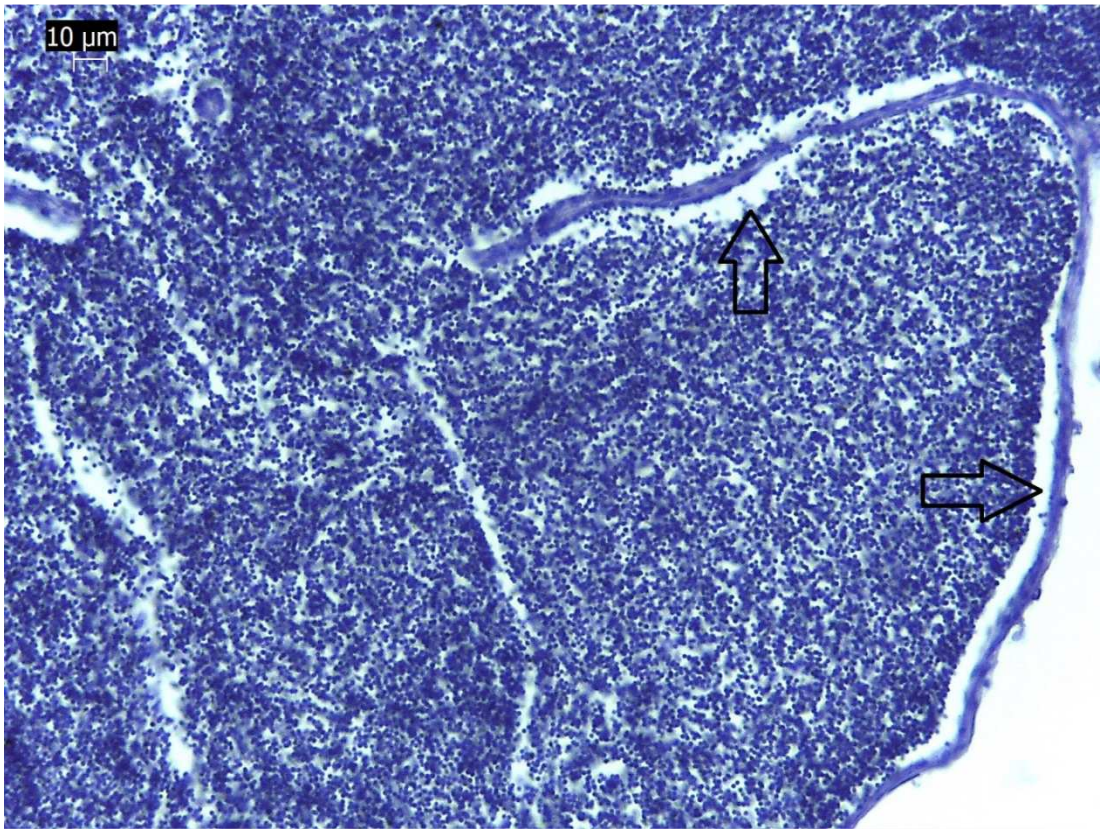

Fig5: Histological section of 30 days cadmium exposed testis of *Rasbora dandia*; Detached basement membrane ⇨

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