International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Mercury Induced Histopathological Changes in the Testis of the Freshwater Fish, *Rasbora dandia*

Priya Rajan[1], Balu T. Kuzhivelil[2]

Applied Biochemistry and Biotechnology Laboratory, Department of Zoology, Christ College, Irinjalakuda, University of Calicut, Kerala-680 125, India

Abstract: ***Aquatic ecosystems are under the constant threat of pollutants released by various anthropogenic activities. Xenobiotic metals like mercury often disrupt normal physiology and homeostasis of organisms. In the present study, reproductively mature freshwater fish, Rasbora dandia were exposed to sublethal concentration of 10 ppb of mercury for 30 days period. Histopathological changes in the testis induced by the mercury are studied. Gonadosomatic index (GSI) of the test fishes is affected by mercury exposure in the present study. Severe deterioration was observed by 30 days of mercury exposure. Other histopathological changes like reduced spermatozoa and proliferation of interstitial tissue are also appeared. The present study implies that chronic mercury exposure induces testis damage in fishes leading to impaired reproductive success.***

Keywords: Histopathology, Mercury, Testis, Gonadosomatic index, *Rasbora dandia*

1. Introduction

Aquatic ecosystems are under the constant threat of pollutants released by various anthropogenic activities. Among aquatic pollutants, mercury is very hazardous as it is highly toxic as well as persistent. Global mercury emissions have natural as well as anthropogenic sources. Human activities make a large contribution to the atmospheric pools of mercury by releasing over 2300 tonnes each year into the atmosphere. Coal combustion is the largest source contributing almost half of the total release (Pacyna et al., 2010).

Mercury is a toxic metal with no known biological role. Properties of bioaccumulation and biomagnification make mercury more hazardous. Xenobiotic metals like mercury often disrupt normal physiology and homeostasis of organisms. One of the most sensitive chronic or sublethal effects of pollutants is on the reproduction (Sprague et al., 1971). Even at low concentration, mercury may affect fish populations through impairment of physiological processes like reproduction (Crump and Trudeau, 2009).

According to Weiner (2013), extend of issues of mercury in fish and wildlife are underestimated and has a scope of substantive scientific discovery. Kidd and Batchelar (2012) pointed out that it is not understood how chronic mercury exposure affects the reproductive success of wild fish. Understandings on modes of action of toxicants make the prediction of their effects as pollutants more accurate (Sprague et al., 1971). Most of the literature regarding the effects of pollutants on the reproduction of fishes is limited to a few teleost fishes like *Clarias batrachus, Channa punctatus, Colisa fasciatus, Heteropneustes fossilis, and Sarotherodon mossambicus* (Pandey, 2000). The present study is our attempt to evaluate the histopathological changes in the testis of native fish *Rasbora dandia*, induced by mercury.

2. Materials and Methods

2.1 Test Organism

R. 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 length between 5.5 to 7 cm length and 1.5 – 4.5 gm were selected and 10 fishes were kept in each aquarium. Fishes were fed daily by standard fish feed.

2.2 Determination of LC_{50}

Mercuric chloride was used as a source of the mercury. To calculate lethal concentration, fishes were exposed to 80, 100, 120, 140 and 160 ppb of mercury. 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 mercury was found to be 133.3 ppb of Hg.

2.3 Exposure Procedure

Experimental fishes were exposed to sublethal concentration of 10 ppb of mercury 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.

2.4 Histological Preparation

Testes were dissected out and placed in 10% formalin for fixation. They 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.

2.5 Histopathology

Histological slides were observed under light microscope and photographed. Control and test sections were compared to analyse histopathological changes induced by mercury.

3. Results and Discussion

According to Saksena (1987) gonadosomatic index is an indicator of the state of the gonads. GSI of the test fishes is affected by mercury exposure in the present study. GSI was found to be slightly declined by 20 days of mercury exposure. By 30 days of mercury exposure considerable reduction in GSI occurred (Table 1). Kirubagaran and Joy (1992) observed a significant decrease in the GSI of male *Clarias batrachus* exposure to the mercurials.

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

Exposure period	Control fishes	Fishes exposed to 10 ppb mercury
10 days	6.3 ± 0.64	6.06 ± 0.389
20 days	6.29 ± 0.14	5.47 ± 0.38*
30 days	6.47 ± 0.12	2.56 ± 0.43**

*Significant at $p<0.05$; ** significant at $p<0.01$

Control testis of *R. dandia* showed germ cells at different stages of maturation. Most abundant among them was spermatozoa. Lobules were filled with large number of spermatozoa with small clusters of spermatids and spermatocytes adjacent to the interstitial tissue (Figure 1). Test fishes showed mercury induced histopathological changes in the testis which gradually increased with increase of exposure period (Figures 1, 2, 3, and 4). Vacant spaces increased inside the lobules by 10 days of mercury exposure (Figure 2). After 20 days mercury exposure interstitial tissue appeared to be proliferated and ripe spermatozoa started declining (Figure 3). Vergilio et al., 2003, reported that mercury induced effects on the testis of *Gymnotus carapo* became more severe with increase of time. They observed that severe deterioration occurred in the testicular histology with congestion of blood vessels and proliferation of interstitial tissue.

On exposure to mercury, severe deterioration was observed by 30 days of mercury exposure in testes of *R. dandia*. Bundles appeared disorganised, vacant spaces enlarged and considerable reduction in spermatozoa occurred (Figure 4). Ram and Sathyanesan (1983) observed that the exposure to mercuric chloride stopped spermatogenesis in teleostean fish *Channa punctatus*. According to Crump and Trudeau (2009) testicular impairment by mercury exposure is attributed to its direct cytotoxic effects as well as disruption of endocrine function. The results of the present study indicate that the presence of mercury in water can induce substantial testicular damage in fishes. Reduction in GSI as well as reduction in the number of spermatozoa indicates reduced reproductive success. Reproductive toxicity of non essential metals like mercury exacerbates their impact on wildlife extinctions. Physiological impacts of mercury on fishes may similarly arise in piscivorous vertebrates as fishes are their food source. Mercury indirectly affects the top predators by reducing food supply while directly affects them by concentrating in the food chain (Crump and Trudeau, 2009).

Vast amounts of anthropogenic mercury have been accumulated in ecosystems, which is only undergoing a slow removal by biogeochemical cycles. Even if anthropogenic emissions are not rising, future deposition of mercury will be increased due to the already accumulated load of mercury. Considerable reduction in primary anthropogenic emissions can only maintain oceanic Hg concentrations at present-day levels (Amos et al., 2013). Rigorous scientific investigation of the sources, consequences and remediation of environmental mercury is necessary (Weiner, 2013). Apart from the controlling of mercury emissions into the environment, the need for constant biomonitoring of pollution prone ecosystems is also rising. Reproductive impairment can be used for devising biomonitoring programmes if sufficient data are provided on the reproductive toxicity of pollutants of major ecological concern.

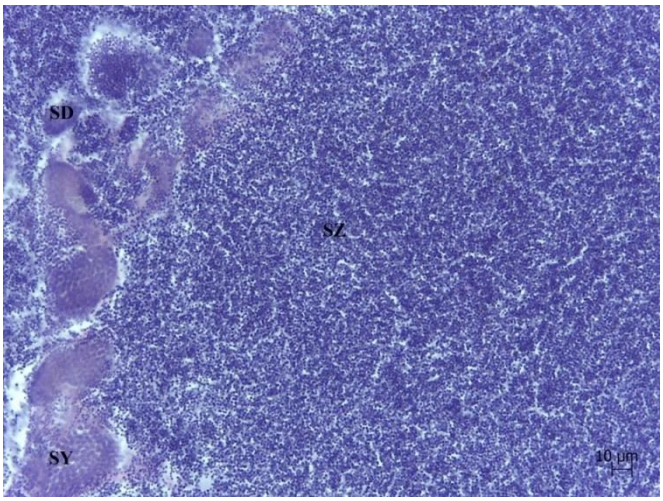

Figure 1: control, SZ- Spermatazoa, SY- Spermatocytes, SD- Spermatids

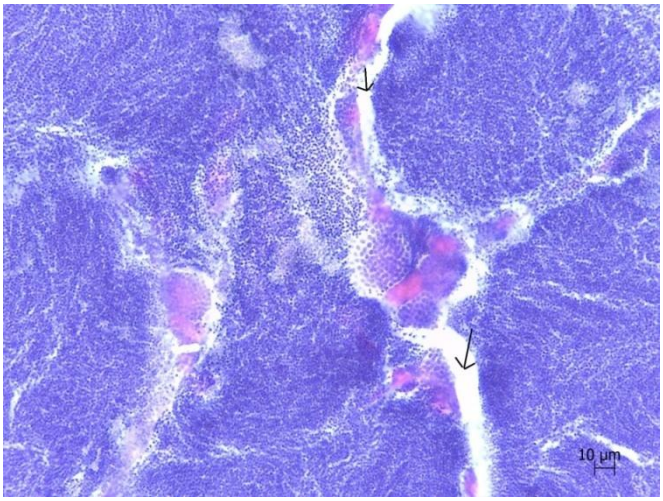

Figure 2: 10 days mercury exposed, Vacant spaces increased (→)

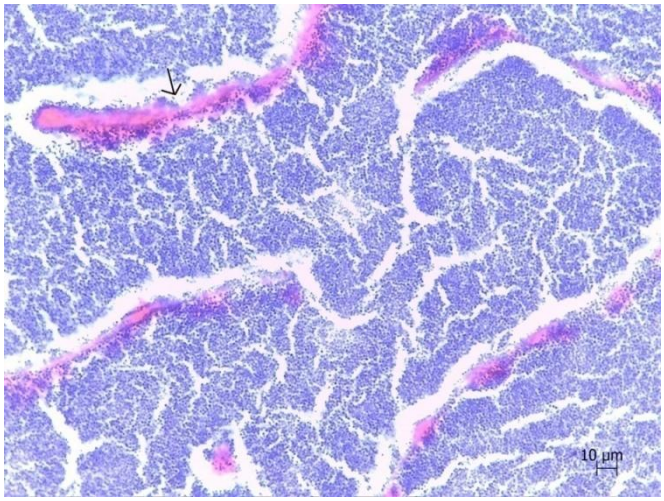

Figure 3: 20 days mercury exposed, proliferation of interstitial tissue (→)

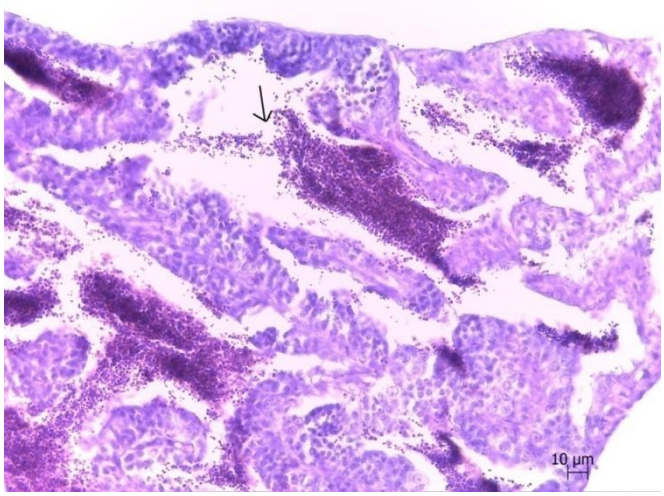

Figure 4: 30 days mercury exposed, reduced spermatozoa (→)

4. Acknowledgements

The first author is indebted to Council of Scientific & Industrial Research (CSIR), India for providing fellowship. We are grateful to Christ College, Irinjalakuda for providing laboratory facilities.

References

[1] Amos, H.M., Jacob, D.J., Streets, D.G., Sunderland, E.M., 2013. Legacy impacts of all-time anthropogenic emissions on the global mercury cycle. Global Biogeochem. Cy. 27, 1–12.
[2] Crump, K.L., Trudeau, V.L., 2009. Mercury-induced reproductive impairment in fish. Environ. Toxicol. Chem. 28, 895–907.
[3] Kidd, K., Batchelar, K., 2012. Mercury. In Homeostasis and Toxicology of Non-Essential Metals (Ed.: Wood C. M. Farrel A. P. and Brauner C. J.). Fish Physiol. 31B, Academic Press, London, UK, pp. 238-284.
[4] Kirubagaran, R., Joy, K. P., 1992. Toxic effects of mercury on testicular activity in the freshwater teleost, *Clarias batrachus*. J. Fish. Biol. 41, 305-315.
[5] Pacyna, E., Pacyna, J., Sundseth, K., Munthe, J., Kindbom, K., Wilson, S., Steenhuisen, F., Maxson, P., 2010. Global emission of mercury to the atmosphere from anthropogenic sources in 2005 and projections to 2020, Atmos. Environ., 4, 2487–2499.
[6] Pandey, A. C., 2000. Aquatic pollution and fish reproduction: a bibliographical review. Indian J. Fish., 47(3), 231-236.
[7] Ram, R. N., Sathyanesan, A.G., 1983. Effect of mercuric chloride on the reproductive cycle of the teleostean fish, *Channa punctatus*. Bull. Environ. Contam. Toxicol. 30, 24–27.
[8] Saksena, D. N., 1987. On the use of gonado-somatic index and volume of the gonads as indicators of gonadal state in India fresh water goby, *Glossogobius giuris* (Ham.) with a note on the role of temperature in fish reproduction. Intl. J. Ichthyol. 8(1), 1-8.
[9] Sprague, J.B., 1971. Measurement of pollutant toxicity to fish: III. Sublethal effects and ‘‘safe’’ concentration. Water Res. 5, 245–266.
[10] Vergilio, S., Moreira, R., Carvalho, C., Melo, E., 2013. Histopathological effects of mercury on male gonad and sperm of tropical fish *Gymnotus carapo* in vitro. E3S Web of Conferences, Volume 1, 2013.
[11] Wiener, J.G., 2013. Mercury exposed: Advances in environmental analysis and ecotoxicology of a highly toxic metal. Environ. Toxicol. Chem. 32, 2175–2178.