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Effect of lead nitrate on the ovaries of the striped snakehead *Channa striatus* (Bloch, 1793)

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ABSTRACT

The effect of sublethal concentrations of lead nitrate (0, 8, 18 and 28 mg l⁻¹) on the ovaries of the freshwater murrel *Channa striatus* (Bloch, 1793) (average length : 20-25 cm; average weight : 50-60 g), randomly distributed (ten fishes per group) into four groups (control, Exp-1, Exp-2 and Exp-3, respectively), were studied in triplicate. Each group of fishes were exposed to lead nitrate for 90 days with the aim to assess the histoarchitectural alterations in the ovaries, at different time intervals. Loosening of connective tissue, deshaped primary oocytes, damaged epithelial layer of oocytes, decreased number of yolk granules in secondary oocytes, upliftment of epithelial layer of secondary oocytes, comparatively decreased number of primary oocytes, necrosis, damaged and irregular shaped oocytes and inflammation were common features in all the three experimental groups exposed to sublethal concentrations of lead nitrate. Severity of the above features increased with increased lead nitrate concentration and duration of exposure.

Keywords: *Channa striatus*, Histoarchitecture, Lead nitrate, Murrel, Ovaries

Heavy metal contamination is an ongoing problem leading to damage of aquatic life, especially fishes and therefore, it has become a major researchable environmental issue of concern (Gill *et al.*, 1990; Hunaiti and Soud, 2000). There are number of routes by which heavy metals enter aquatic habitat causing cytotoxic, mutagenic and carcinogenic effects in aquatic organisms (More *et al.*, 2003; Thirumavalan, 2014). Lead is abundantly found in the earth's crust and has widespread industrial applications (Palaniappan *et al.*, 2008). Once introduced into the aquatic ecosystem lead causes severe intimidation to aquatic life and it is notorious to cause severe histological and metabolic alterations in fishes. Histopathological and histoarchitectural changes are being widely used as reliable indicators to study the health of fishes exposed to contaminants (Wester and Canton, 1991; Hinton *et al.*, 1992; Schwaiger *et al.*, 1997; Thophon *et al.*, 2003; Dar and Jha, 2013). Histoarchitectural changes emerge as a medium-term response to sub-lethal stressors and histology also provides a quick technique to identify effects of pollutants in various tissues of organisms (Johnson *et al.*, 1973; Dar *et al.*, 2014). Therefore, the present study was designed with the aim to investigate histoarchitectural changes induced by chronic exposure to lead nitrate in the ovary of the freshwater murrel *Channa striatus* (Bloch, 1793).

Live specimens of *C. striatus* (length 20-25 cm and weight 50-60 g) were collected from different fish markets in Bhopal, Madhya Pradesh and acclimatised to laboratory conditions for a period of 15 days prior to the experiment. Fishes were divided into four groups of 10 fishes each (Control, Exp-1, Exp-2 and Exp-3) and were exposed to sublethal concentrations (0, 8, 18 and 28 mg l⁻¹ respectively) of lead nitrate (Ranbaxy India Ltd.), in triplicate for a total period of 90 days. Exposure concentration was decided on the basis of 96 h LC₅₀ value of lead nitrate. The median lethal concentration (LC₅₀) values at 95% confidence limits for different exposure period were calculated using the software "Trimmed Spearman Karber method", version-1.5 (Hamilton *et al.*, 1977). The LC₅₀ value was observed to be 284.3 mg l⁻¹. To maintain desired lead nitrate concentration throughout the experimental duration of 90 days, water in each aquarium tank was changed on every alternate day. At regular intervals of 30 days (at the end of 30th, 60th and 90th days of experimentation), three fishes from each group were sampled and the ovaries were dissected out and fixed in aqueous Bouin's fixative for 48 to 72 h (Luna, 1992). After fixation, the tissues were processed (Luna, 1992), embedded in paraffin wax and 5-6 µm thick sections were cut with the help of rotatory microtome and

stained with Ehrlich's haematoxylin and eosin (H&E) for histopathological examinations. The sections were examined under a trinocular compound microscope (Olympus) and photomicrographs were taken.

Histological sections of the ovaries of the experimental fishes are shown in Fig. 1 (a-j). Histological section of the ovaries of control fish showed normal architecture, characterised by ovigerous lamellae having both primary and secondary oocytes (Fig. 1a). Increased histoarchitectural alterations were observed in the ovaries of experimental fishes in proportion with increase in concentration as well as period of exposure to sublethal levels of lead nitrate.

Experimental fishes in the group, Exp-1 exhibited loosening of connective tissue, irregular shaped and deshaped primary oocytes on the 30th day of lead nitrate exposure (Fig. 1b), clustering of oocytes and damaged epithelial layer of oocytes on the 60th day (Fig. 1c) and loosening of connective tissue, damaged secondary oocytes and mild inflammation on the 90th day of exposure (Fig. 1d).

In Exp-2 fishes, clustering of oocytes, mild loosening of connective tissue and damaged epithelial layer of primary oocytes on 30th day (Fig. 1e), loosening of connective tissue, irregular shaped oocytes, indistinguishable secondary oocytes, damaged epithelial layer of primary and secondary oocytes and damaged oocytes on 60th day (Fig. 1f) and loosening of connective tissue, comparatively decreased number of primary oocytes, necrosis, damaged epithelial layer of oocytes, damaged and irregular shaped oocytes as well as inflammation on the 90th day of lead nitrate exposure (Fig. 1g) were observed.

The common histoarchitectural changes observed in case of Exp-3 fishes on the 30th day of lead nitrate exposure were loosening of connective tissue, clustering of oocytes, irregular shaped oocytes and damaged epithelial layer of primary oocytes (Fig. 1h). The alterations were more severe as extensive loosening of connective tissue, thickening and damaged ovarian wall, comparatively less number of oocytes along with severe inflammation, necrosis, damaged oocytes, decrease in the number of yolk granules and large interfollicular space on the 60th and 90th days of exposure (Fig. 1, i and j respectively).

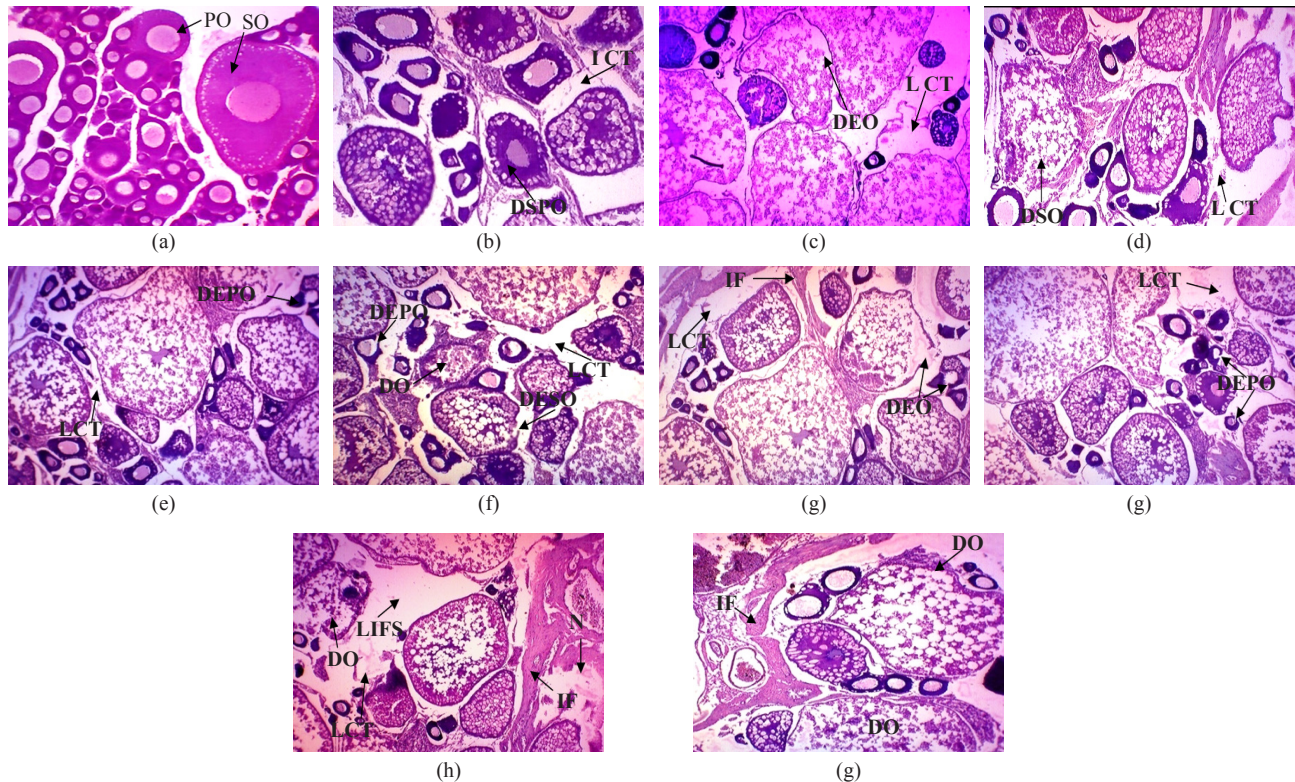


Fig. 1. Histological sections of ovary of *C. striatus* (a) Control group, (b) Exp-1 group after 30 days, (c) Exp-1 group after 60 days, (d) Exp-1 group after 90 days, (e) Exp-2 group after 30 days, (f) Exp-2 group after 60 days, (g) Exp-2 group after 90 days, (h) Exp-3 group after 30 days, (i) Exp-3 group after 60 days, (j) Exp-3 group after 90 days.

LCT - loosening of connective tissue, DSPO - deshaped primary oocytes, DEO - clustering of oocytes and damaged epithelial layer of oocytes, IF - inflammation, DEPO - damaged epithelial layer of primary oocytes, DEO - damaged epithelial layer of oocytes, DO - damaged oocytes, N - Necrosis, LIFS - large interfollicular spaces (H&E; X 100)

Several authors have reported toxicological changes in the ovaries of fish exposed to heavy metals. Adeymo (2008) observed similar results in *C. gariepinus* exposed to different concentrations of lead nitrate for a period of eight weeks. Disruption of follicular lining around the oocytes, damaged stroma, less number of pre-vitellogenic and vitellogenic oocytes were observed by Jaat *et al.* (2013) due to chronic exposure to mercuric chloride for 60 days in the ovary of *C. gariepinus*. Masarat *et al.* (2014) reported damaged follicular lining, deformed oocytes, clumping of primary oocytes, damaged oocytes and reduction in the number of oocytes along with necrosis in the ovaries of *C. gariepinus* on exposure to 0.008 mg l⁻¹ of mercuric chloride for 60 days. It is clear from the present study that the ovaries of *C. striatus* are affected by even a sublethal dose of 8 mg l⁻¹ of lead nitrate, while most severe damage occurred on the 60th and 90th day of exposure to 28 mg l⁻¹ lead nitrate.

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