Histophysiological responses in ovary and liver of *Cyprinus carpio* after short term exposure to safe concentration of mercuric chloride and recovery pattern

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**Abstract:** Effect of short term (7 days) exposure to safe concentration of HgCl$_2$ (0.5 ppm) and changes 7 days after withdrawal of the treatment on histophysiology of ovary and liver in yearlings of *Cyprinus carpio* were assessed during active phase of reproductive cycle. Noticeable degenerative histophysiological changes were observed in both ovary and liver after exposure which were more prominent in the group with abnormal behaviour. After withdrawal of the HgCl$_2$ treatment the recovery was apparent in both organs but was more appreciable in liver. These observations indicated that even safe concentration of HgCl$_2$ might not be fully devoid of deleterious influence on reproductive functions in *Cyprinus carpio*.

**Key words:** *Cyprinus carpio*, Mercuric chloride, Toxicity, Ovary, Liver, Recovery

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**Introduction**

Water pollution is caused by various forms of mercury i.e. methyl mercury, mercuric chloride, phenyl mercuric acetate etc. and which are more dangerous because of their high toxicity, non-biodegradability and biomagnification tendencies. These different forms of mercury including mercuric chloride (HgCl$_2$) adversely affect the reproductive processes at different levels in fishes (Kime, 1995; Jagadeesan and Pillai, 2007). Mercury in *Cyprinus carpio* (Alam and Maughan, 1995) and HgCl$_2$ in *Channa punctatus* (Patil and Dhande, 2000) and *Notopterus notopterus* (Sindeh et al., 2002) were reported to be toxic. Adverse effects of HgCl$_2$ on testicular recrudescence (Masud et al., 2001), first ovarian maturity (Masud et al., 2003), behavioural and hematological responses (Masud et al., 2005) have also been reported in *C. carpio*. In most of the studies only deleterious effects in response to HgCl$_2$ exposure have been reported but apparently no effort has been made for assessing the recovery response after withdrawal of the treatment in any fish. In the present study an attempt has been made to assess the recovery response 7 days after withdrawal of treatment in ovarian and liver histophysiology of *C. carpio* after exposure to HgCl$_2$ for 7 days.

**Materials and Methods**

The present experiment was conducted during last week of December to first week of January in female yearlings of *C. carpio* in the active phase of their reproductive cycle. The healthy specimens of *C. carpio* having average weight of 90 g (75-100 g) and average length 12 cm (10-15 cm) were obtained from the Instructional Fish Farm of the College and were acclimated in flow-through rectangular cemented tanks (2 m x 1 m x 1 m) in ambient light and temperature conditions. During acclimation fish were fed with conventional feed (rice bran and oil cake in 1:1 ratio) at the rate of 10% of the body weight. After proper acclimation they were divided into 2 groups of 18 fish each. One group was treated with HgCl$_2$ (0.5 ppm) (Masud et al., 2005) for 7 days, while other group served as control. Observations were made twice daily for mortality and other behavioural changes. During this experiment water was changed on alternate day and after feeding fresh dose of HgCl$_2$ was added.

After 7th day of treatment with HgCl$_2$, five stressed and five normal specimens were sacrificed. Liver and ovary were dissected out and fixed in Bouin’s fixative. Remaining fish of treated and control groups were kept in flowing clean water for another week for recovery response and then these fish were also sacrificed and their ovary and liver were also fixed in Bouin’s fixative. The fixed tissues were processed for cutting transverse sections at 5 µm which were stained with haematoxylin and eosin (H&E) for microphotography.

**Results and Discussion**

**Histopathological changes in ovary:** The control fish ovaries exhibited characteristics of ovarian development such as the presence of large number of oogonials and immature oocytes along with moderate number of vitellogenic and yolky oocytes (Fig. 1). Vitellogenic oocytes were in the active state of vitellogenesis as prominent yolk bodies were present in them (Fig. 2). The oogonial cells were also at the active phase of proliferation as they had prominent nuclei (Fig. 3). In fish exposed to toxicologically safe concentration (0.5 ppm) of HgCl$_2$ showing normal behaviour (Masud et al., 2005), the ovaries showed inhibition of early vitellogenesis as they were full of concurrently degenerating oogonials and immature oocytes (Fig. 4). The degenerative changes in oogonials and immature oocytes included the ooplasmic and nuclear dissolution resulting in the presence of debris (Fig. 5). In the group of HgCl$_2$ exposed fish showing abnormal behaviour (Masud et al., 2005) the...
Fig. 1: Ovary of control C. carpio - oogonials (►), immature (►), vitellogenic (►►) and yolky oocytes (►►). HE X 45
Fig. 2: Vitellogenic oocyte with yolk material (►►) and germinal vesicle (GV) in control. HE X 225
Fig. 3: Ovary of control - differentiated (►►) and differentiating (►) oogonials. HE X 450
Fig. 4: Oogonials (►), immature (►), dissoloving (►►) oocytes and debris in ovary of treated fish with normal behavior. HE X 45
Fig. 5: Debris (►►), oogonials (►) and immature oocytes (►►) in ovary of treated fish with normal behavior. HE X 450
Fig. 6: Oogonial nest (►), debris of degenerated oogonials (►►), normal immature oocyte (►►), necrotic regions (N) within hepatic tissue (H) in liver of treated fish with abnormal behavior. HE X 45
Fig. 7: Necrotic nuclei of hepatocytes (►►) and ooplasmic degeneration of immature oocyte (►►) in treated fish. HE X 450
Fig. 8: Ovary (control) after 14 days - immature (>), early (→) and late (≥-) vitellogenic oocytes. HE X45
Fig. 9: Intra-vesicular (> and inter-vesicular (≥-) yolk materials in interior and exterior regions of oocytes. HE X225
Fig. 10: Normal oogonials (> and immature oocytes (≥-) in ovary after recovery. HE X45
Fig. 11: Normal multi-nucleolar stage immature oocyte (> and active primordial cells (≥-) in ovary after recovery. HE X450
Fig. 12: Liver (control) - active hepatocytes (> and hepatopancreatic cells (≥-) HE X450
Fig. 13: Degenerating hepatocytes (> and hepatopancreatic cells (≥-) with debris (►) in liver of treated fish with normal behavior. HE X450
Fig. 14: Hypertrophied hepatocytes (> and hepatopancreatic (≥-) cells in liver of treated fish with abnormal behavior. HE X450
Fig. 15: Normal hepatocyte (> and hepatopancreatic (≥-) cells in liver (control) after recovery. HE X450
Fig. 16: Active hepatocyte (> and hepatopancreatic (≥-) cells with cytoplasmic vacuolization in liver of recovery group. HE X450
Ovarian components embedded in hepatic tissue exhibited variable degree of deleterious changes (Fig. 6). In their ovaries also most of the oogonial cells and immature oocytes were in the process of degeneration particularly at the ooplasmic level and these were devoid of vitellogenic oocytes. The huge mass of debris of such dissolved ovarian and hepatic components could be seen prominently in them (Fig. 7). The ovaries in control group exhibited further advancement of oocyte development after 14 days of the start of experiment with the presence of early and late vitellogenic oocytes along with large number of immature oocytes (Fig. 8, 9).

Observations in the present study indicated over-all reduction in oocyte development in ovaries of fish exposed to safe concentration (0.5 ppm) of HgCl$_2$. Presence of large number of oogonials and immature oocytes and a few early degenerating vitellogenic oocytes with complete dissolution of ooplasmic and nuclear materials in ovaries of treated fish showing normal behaviour, absence of early vitellogenic oocytes and variable degree of degenerative changes in oogonials and immature oocytes leading to huge debris mass of their dissolved components in fish with abnormal behaviour were indicative of variable responses of treatment with same concentration of HgCl$_2$ on this fish. Deposition of mercuric chloride and significant decrease in RNA/DNA ratio after 9 days of exposure was reported in prespawning ovary of Labeo rohita (Aditya et al., 2002). Large degenerative changes in ovary and liver leading to decreased GSI and complete mortality of C. carpio were observed after 45 days exposure to 0.5 ppm HgCl$_2$ (Masud et al., 2003). Histopathological changes in tests, ovary and liver of C. carpio exposed to 0.1 ppm HgCl$_2$ for 45 or 60 days revealed that even this level of exposure to it was not fully free from deleterious consequences (Masud et al., 2001, 2003). Deleterious effect of HgCl$_2$ at 0.5 and 0.1 ppm levels during 124 hr exposure was apparent on body colour and hematological responses in C. carpio (Masud et al., 2005). Acute toxicity of HgCl$_2$ on the fish, Anabas testudineus during 24 to 96 hr exposure (Sinha and Kumar, 1992) and adverse effects on haematological changes in C. batrachus (Joshi et al., 2002) were also reported. These multifarious deleterious effects of HgCl$_2$ at different dose levels on various physiological parameters of different fish species including C. carpio during short or long term exposures indicated that it is highly toxic even at low doses.

**Histopathological changes in liver:** The liver of control fish had its normal foamy appearance having active hepatocytes with prominent nuclei and round nucleoli. The cytoplasmic vacuolization and granulation suggested occurrence of active hepatic biosynthesis. The exocrine hepatopancreatic cells containing large round nuclei with prominent nucleoli also exhibited biosynthetic activities. (Fig. 12). In the HgCl$_2$ treated group of fish with normal behaviour, the hepatocytes were inactive and most of them exhibited nuclear pyknosis and necrosis, evident by the presence of debris. The exocrine hepatopancreatic cells revealed various degree of degeneration as they contained the debris and clumped darkly stained necrotic material (Fig. 13). In the HgCl$_2$ treated fish with abnormal behaviour though the hepatocytes and the exocrine hepatopancreatic cells exhibited hypertrophy but these were accompanied by nuclear pyknosis and necrosis attributable to acute stress exposure (Fig. 14). Level of degenerative changes in hepatocytes and hepatopancreatic cells of treated fish compared to control group indicated that effect of HgCl$_2$ exposure was slightly acute on liver compared to ovaries. Similar degenerating changes were observed in liver of both sexes of C. carpio after exposure to HgCl$_2$ at 0.1 ppm for 45 and 60 days (Masud et al., 2001, 2003) and in its female at 0.5 ppm after 45 days (Masud et al., 2003). Decreased glucose-6-phosphatase and hexokinase activity was reported in C. punctatus after exposure to sublethal concentration of HgCl$_2$ for 120 days (Sastry and Rao, 1984). However, despite being main organ responsible for breaking down toxicants entering fish body such studies on liver are very few.

**Recovery responses in ovary and liver:** In recovery group the ovaries showed recovery response up to some extent in the term of recruitment of normal immature oocytes possibly from the group of those oogonials which were not affected by exposure to HgCl$_2$ (Fig. 10, 11). However, the presence of debris in the proximity of normal immature oocytes indicated the persistence of HgCl$_2$ toxicity even after withdrawal of the treatment.

The liver and hepatopancreatic cells of control fish exhibited normal histological appearance at the time of termination of recovery experiment (Fig. 15). The hepatocytes of recovery group exhibited cytoplasmic vacuolation and granulation with prominent nuclei suggesting resumption of the normal biosynthetic activities almost to the level of control group. The exocrine cells of hepatopancreas with larger nuclear and nucleolar sizes also showed the sign of regeneration (Fig. 16).

Recovery response in ovary of C. carpio after 7 days withdrawal of HgCl$_2$ treatment was limited to recruitment of immature oocytes possibly from unaffected oogonials. However, presence of debris material was indicative of irrevocable damage in ovary. The recovery shown by liver histopathology reached to the normal level of control with regard to vacuolization and granulation of hepatocytes having prominent nuclei. Regeneration of hepatopancreatic cells, as evident by larger nuclear and nucleolar sizes, indicated that recovery response at liver level was better compared to ovary after withdrawal of HgCl$_2$ treatment. Observations on similar recovery response are apparently not available for HgCl$_2$ or other pollutants in any fish except for carbofuran and cypermethrin in L. rohita in which prominent recovery in both treated groups and faster recovery with carbofuran was reported (Sarkar et al., 2005). These observations on C. carpio after exposure and withdrawal of HgCl$_2$ for 7 days indicated that deleterious changes and recovery responses of ovary and liver varied in a tissue specific manner.

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References


