Microcystin toxicity in a freshwater fish, *Heteropneustes fossilis* (Bloch)

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Microcystin is a toxin secreted by cyanobacteria, which are the dominant phytoplankton in eutrophic freshwater bodies. The present study reports histopathological, hematological and biochemical changes in the freshwater fish, *Heteropneustes fossilis* due to toxicity of microcystin. Toxic effects were observed in liver, kidney, corpuscles of Stannius, gills and gonads. Bilirubin concentration increased in the blood of treated fish. Concentration of cholesterol, triglycerides, aspartate aminotransferase and alanine aminotransferase, and alkaline phosphatase was affected due to toxicity. It is suggested that microcystin concentration should be monitored in the drinking water and the public awareness should be created.

Keywords: Cyanobacteria, freshwater fish, microcystin, toxicity.

CYANOBACTERIA are the dominant phytoplankton group in eutrophic freshwater bodies[1]. They are prokaryotes possessing cell walls composed of peptidoglycon and lipopolysaccharide layers instead of cellulose of green algae[2]. All cyanobacteria are photosynthetic and possess chlorophyll *a*. Morphological diversity ranges from unicells to small colonies of cells to simple and branched filamentous forms[3].

Cyanobacteria are capable of producing two kinds of toxin, cyclic peptide hepatotoxin and alkaloid neurotoxin. Serious illness such as hepatoenteritis, a symptomatic pneumonia and dermatitis may result from consumption of or contact with water contaminated with toxin-producing cyanobacteria.

Microcystins are a family of toxins produced by different species of freshwater cyanobacteria, namely *Microcystis* (order Chroococcales), *Anabaena* (order Nostocales), and *Oscillatoria* (order Oscillatoriales). Microcystins are monocyclic heptapeptides composed of D-alanine at position 1, two variable L-amino acids at positions 2 and 4, γ-linked D-glutamic acid at position 6, and three unusual amino acids; β-linked D-*erythro*-β-methylaspartic acid (MeAsp) at position 3; (2S, 3S, 8S, 9S)-3-amino-9-methoxy-2,6,8 trimethyl-10-phenyldeca-4, 6-dienoic acid (Adda) at position 5 and *N*-methyl dehydroalanine (MDha) at position 7. There are over 50 different microcystins that differ primarily in the two L-amino acids at positions 2 and 4, and methylation/demethylation on MeAsp and MDha. The unusual amino acid Adda is essential for the expression of biological activity. Other microcystins are characterized largely by variation in the degree of methylation; amino acid 3 has been found to be D-aspartic acid, replacing β-methylaspartic acid and amino acid 7 to be dehydroalanine, replacing *N*-methyldehydroalanine[4,5]. The most common microcystin is microcystin-LR, where the variable L-amino acids are leucine (L) and argenine (R). The present work has been taken to the study of the effects of *Microcystis* toxin on fish.

Microcystis aeruginosa was collected from Sagar Lake (Sagar is located at 25°51′N lat. and 78°45′E long.) and filtered on filter paper. Cells were dried at room temperature and stored at 8°C. The process of isolation of toxins was carried out as described by Harada *et al.*[6]. Ten grams of dried cells was extracted with 100 ml of methanol and water (70 : 30). The mixture was sonicated three times for 5 min. Total extract was frozen overnight and then centrifuged. The supernatant was separated and filtered through Whatmann No. 1 filter paper. The filtrate was concentrated on Octadecil–Silane cartilage (C18), which was washed with 20 ml of distilled water, followed by 20 ml of 20% methanol and eluted with 20 ml of 100% methanol. The extract was completely dried by evaporation and a 10% solution of extracted microcystin solution was made in saline water. *Heteropneustes fossilis* was selected for the toxicity study. The fishes used in the experiment were collected from the local fish market. The average weight of the fish used in the experiment was 60 to 70 g.

The fishes were kept at room temperature for 1 week. Then 0.1 ml (5 units) and 0.2 ml (10 units) of 10% solution of microcystin was injected intraperitoneally just above the caudal fin (LC_{50} 0.3 ml and LC_{100} 0.4 ml was determined experimentally). The fishes could survive at this concentration. Two types of experiments were performed to study the effect of microcystin on the fishes: (i) Single sub-lethal dose of short term and (ii) Double sub-lethal dose of long term. In single sub-lethal dose, a single dose was given to the fishes and they were sacrificed after 1, 3, 6, 24, 72 and 102 h. In the second experiment after the first dose, a second dose was given after 3 days, and the fishes were sacrificed after 5, 10, 15, 20 and 25 days. Histopathological changes due to *Microcystis* toxin were examined. Fish tissues (liver, kidney, gonads and gills) were fixed in aqueous Bouin's fluid. Then 5 μ sections were stained by Haematoxilin and Eosin. Blood was taken out from the caudal area using a syringe. Enzymatic studies were performed with enzyme kits of Ames SEAC, Miles India Ltd and analysed on R.A.50 Chemistry Analyzer.

Histopathological changes due to microcystin toxicity in the liver mainly include architectural changes in the liver, hepatocytes swelling, dissociation of hepatocytes, hepatocytes showing pycnotic nuclei, broken sinusoidal endothelium, ruptured blood vessels with haemorrhage and vacuoles in the haepatocytes (Figure 1 *a–f*). In mammals, the *Microcystis* toxin causes extensive haepatic hemorrhage and general necrosis in the liver[7–10]. Rabergh *et al.*[11] re-

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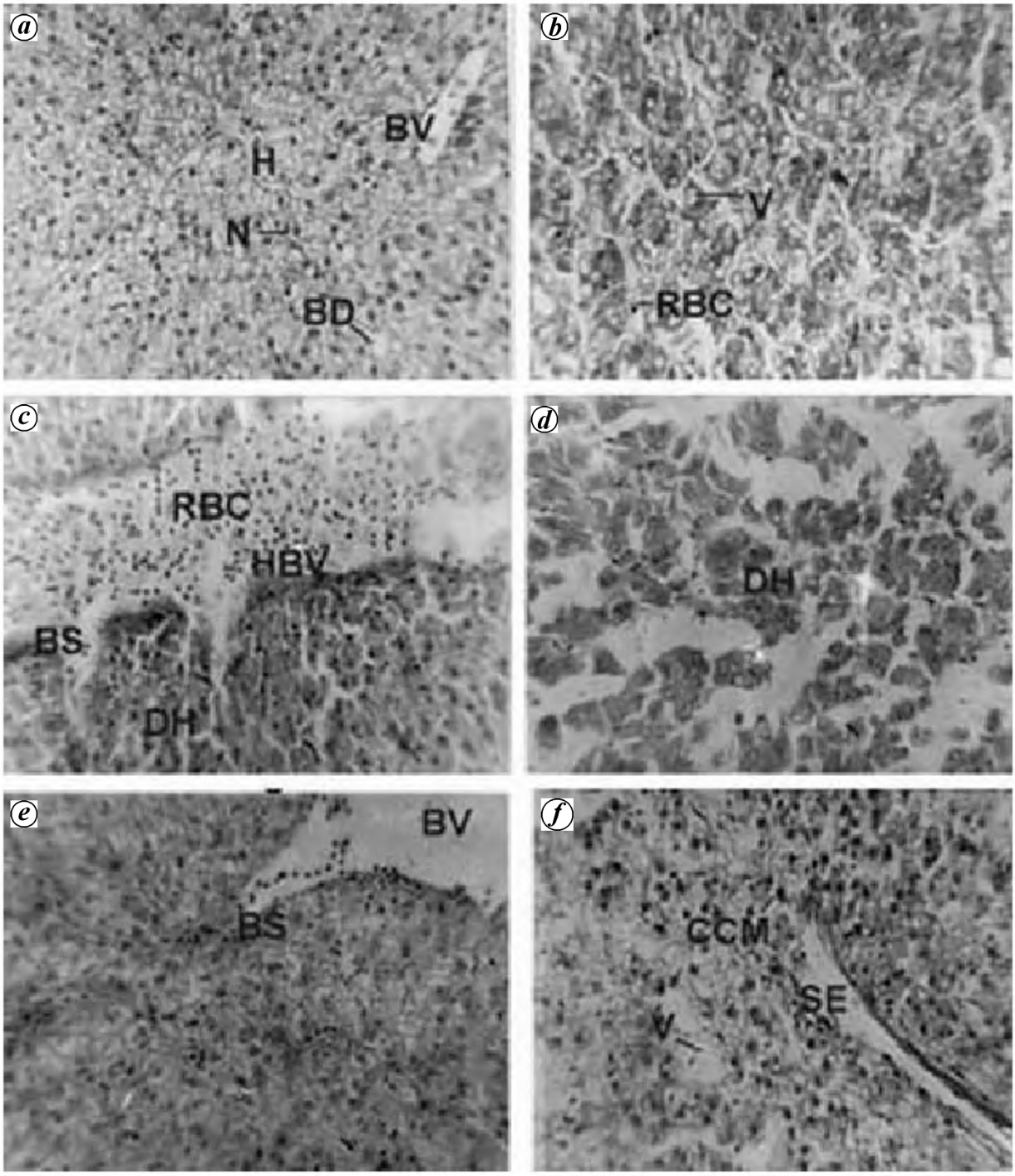

Figure 1. Section of liver of *Heteropneustes fossilis*. ***a***, Control group showing normal nucleus and hepatocytes. H, Hepatocyte; BD, Bile duct; N, Nucleus; BV, Blood vessel; 10 × 45 (H&E stain). ***b***, Experimental group (0.1 ml: 1 h after treatment) showing vacuole in cytoplasm and small nucleus in hepatocyte. RBC, Red blood cell; V, Vacuoles; 10 × 45 (H&E stain). ***c***, Experimental group (0.1 ml: 72 h after treatment) showing dissociated hepatocytes and haemorrhagic blood vessels. HBV, Haemorrhagic blood vessel; BS, Blood seeping; DH, Dissociated hepatocytes; 10 × 45 (H&E stain). ***d***, Experimental group (0.2 ml: 1 h after treatment) showing pycnotic nuclei and vacuoles in cytoplasm of hepatocyte; 10 × 45 (H&E stain). ***e***, Experimental group (0.2 ml: 6 h after treatment) showing sinusoidal spaces filled with blood and pycnotic nuclei in hepatocyte; 10 × 45 (H&E stain). ***f***, Experimental group (0.2 ml: 5 days after treatment) showing swelling in hepatocytes and condensed chromatin in nuclei. CCM, Condensed chromatin material; SE, Sinusoidal endothelium; 10 × 45 (H&E stain).

ported that the hepatocytes were markedly swollen with granular appearance of the cytoplasm. Fishes injected with higher dose (250 and 300 μg/kg) showed areas with disrupted parenchymal architecture and necrosis. They reported reduction in glycogen content in the liver after staining the section using PAS staining method. The sequence

of hepatic cell death, with necrosis appearing very early and apoptosis rather late in the development of the liver damage has also been observed in mice treated with acutely toxic doses of microcystin[10].

Tencalla and Dietrich[12] have reported the effect of *Microcystis* gavage in rainbow trout *Oncorhynchus mykiss*. The typical chord structure of trout liver disappeared and cytoplasm of hepatocytes was condensed. Between 3 and 12 h, the loss of structure increased across the tissue and limited punctual microhaemorrhaging via ruptured vessels was observed. The hepatocyte nuclei began to condense and cytoplasm of these cells was highly vacuolated.

Histopathological examination of the liver of *H. fossilis* after short-term and long-term treatment of microcystin clearly shows that the parenchymal architecture of the liver is disturbed and hepatocyte show dissociation after 3 h of treatment. In the later stage, e.g. after 24 h of treatment, the hepatocyte appears swollen and cytoplasm appears granular. The hepatocyte nuclei become pycnotic. The sinusoidal endothelium breaks down after 3 h of treatment and blood comes out from the ruptured vessels. Haemorrhage was seen in the 24-h treatment stage. In long-term treatment, the effect become more prominent. The hepatocyte cells show condensed chromatin, which is a characteristic apoptosis cell. After 15 days of treatment in liver, apoptotic cells appear. Blood capillary endothelium ruptured and blood spilling into the liver tissues was observed.

Intraperitoneal injection of microcystin causes gill damage in *H. fossilis*. Microcystin reaches the gill through blood circulation, although a lesser amount can reach the gills because the kidney filters some of it and some amount is stored in the liver before it reaches the gills. This is the reason why the gills are less affected compared to the liver and kidney.

The first change observed was the enlargement of the epithelial cells. The gills were swollen compared to the control. After 1 h of treatment, lifting of the epithelial lining was observed. The mucous cells and chloride cells were large and prominent. However, at a later stage, proliferation in the chloride cells was observed. Edematory spaces were formed between layers of the epithelium. Endothelial cells of the capillaries were also affected and at many places the capillaries were damaged and blood spilled into the space (Figure 2 *a–f*). In the ten-days stage, necrotic and apoptotic cells were seen. In 15 to 20 days after treatment, the epithelial lifting reduced and gills showed repairing of the damaged structure.

Histopathological changes in the gills of fishes due to microcystin intoxication have been less reported. Carbis *et al.*[13] have reported that 37% of the fishes had gills with pinpoint necrosis, epithelial ballooning, folded lamellar tips and exfoliation of the lamellar epithelium compared to carp (*Cyprinus carpio*) from Lake Wellington. In the present work also similar observations were made: Epithelial lifting, necrosis, lamellar fusion, hyperplasia, lamellar aneurism, hypertrophy, chloride cell damage, apoptosis in the cells, lamellar blood dilation, blood spill over to the edematous space at different stages of dose treatment.

The effect of microcystin on plasma cortisol, glucose levels plasma Na^+ and Cl^- concentration, and Na^+/K^+-ATPase activity has been reported[14,15]. In the gills of freshwater fish, Ca^{2+} influx is facilitated by a Ca^{2+} channel in the apical membrane of chloride cells[16]. Extrusion of Ca^{2+} from the cell across the basolateral membrane is mediated by a high-affinity Ca^{2+}-ATPase and possibly by Na^+/Ca^{2+} exchange[17,18]. This extrusion process could regulate the intracellular Ca^{2+} concentration by Ca^{2+}-binding proteins, by active Ca^{2+} uptake into endoplasmic reticulum[19] or sequestration by mitochondria via a uniporter[20,21]. Inhibition of Ca^{2+}-ATPase of the basolateral membrane of chloride cells[16] Cd^{2+} causes hypocalcaemia[22], indicating the importance of this branchial Ca^{2+} pump in calcium homeostasis.

In the present study it was observed that microcystin affects the chloride cells and thus it can be concluded that, directly or indirectly, the branchial Ca^{2+} pump is disturbed resulting in the calcium homeostasis. Bury *et al.*[15] have reported that strains of cyanobacterium *Microcystis aeruginosa* produce compounds that inhibit Ca^{2+} uptake and gill K^+-dependent PNPPase activity in *Tilapia*.

The effect of microcystin on the histology of kidney has been reported in fish and rat. Rabergh *et al.*[11] have reported that in the kidney, Bowman's capsules of the glomeruli were dilated in fish injected with 250 and 300 μg/kg microcystin. These changes were apparently more severe than the relatively mild effects on kidney cells observed in microcystin toxicosis in mice[23]. Vajcova *et al.*[24] have reported large dystrophic and neurobiotic alteration of hepatocytes, as well as kidney tubuli after intraperitoneally applied pure microcystin in silver carp. Kotak *et al.*[25] have reported hepatic renal pathology of intraperitoneally administered microcystin-LR in rainbow trout (*Oncorhynchus mykiss*). Kidney lesions in fish consisted of coagulative tubular necrosis with a dilation of Bowman's spaces.

Fischer and Dietrich[26] have reported the effects of microcystin in the kidney and hepatopancrease of *Cyprinus carpio*. Damage of renal proximal tubular cells and hepatocytes was observed at early as 1 h. Milutinovi *et al.*[27] reported acute toxic effects of high doses of mononuclear cells in rat kidney. The glomeruli were collapsed and filled with eosinophilic materials. The tubuli of the outer and inner medulla were dilated with lumen-filled eosinophilic proteinaceous casts.

In the present study, similar observations were made after intraperitoneal injection of the extract of the *Microcystis* in freshwater catfish, *H. fossilis*. Histopathological observations show that 3 h after treatment, the effects start appearing in the kidney. The renal tubules and cells were greatly affected. In the late stages, the renal tubule cells show necrosis. Vacuoles appear in the cytoplasm of the cells. Glomeruli show degeneration in the late stages. Bowman's capsule shows dilation. The kidney tissue

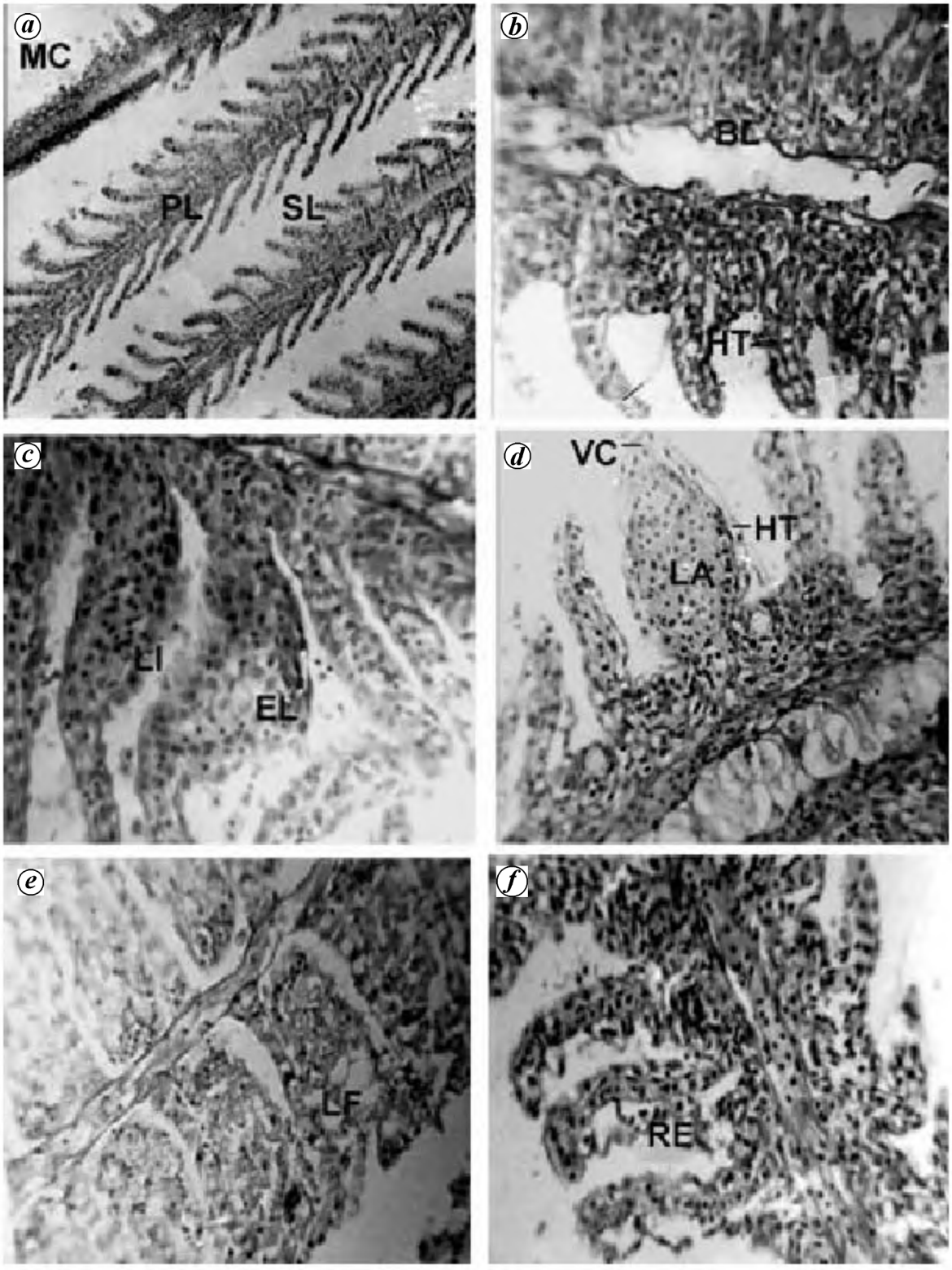

Figure 2. Section of gill filament of *H. fossilis*. ***a***, Control group showing straight gill lamellae with compact cellular structure and large number of mucus cells at the tip of primary lamellae. MC, Mucus cell; PL, Primary lamellae; SL, Secondary lamellae; 10 × 10 (H&E stain). ***b***, Experimental group (0.1 ml: 3 h after treatment) showing epithelial lifting and hypertrophy in the secondary lamellae. BL, Basal lamina; HT, Hypertrophy; 10 × 45 (H&E stain). ***c***, Experimental group (0.1 ml: 6 h after treatment) showing leukocyte infiltration and epithelial lifting. LI, Leukocyte infiltration; EL, Epithelial lifting 10 × 45 (H&E stain). ***d***, Experimental group (0.1 ml: 5 days after treatment) showing hypertrophy in epithelial cells, lamellar aneurism and vascular congestion. VC, Vascular congestion; LA, Lamellar aneurism 10 × 45 (H&E stain). ***e***, Experimental group (0.2 ml: 1 h after treatment) showing lamellar fusion and blood spill into lamellae and gap between basal lamina and gill ray. LF, Lamellar fusion; 10 × 45 (H&E stain). ***f***, Experimental group (0.2 ml: 10 h after treatment) showing ruptured epithelium, dilated blood sinus. RE, Ruptured epithelium; 10 × 45 (H&E stain).

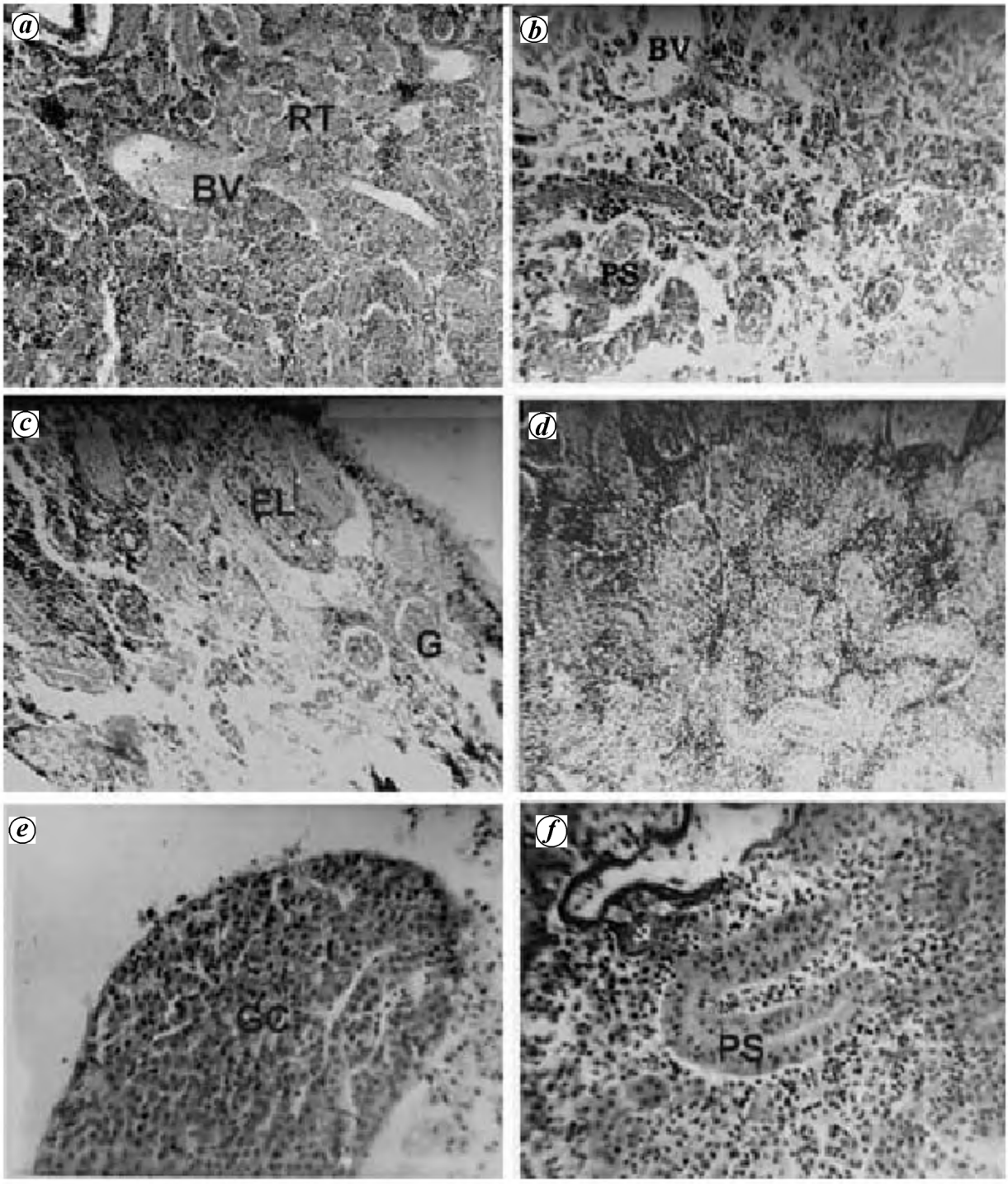

Figure 3 ***a–d.*** Section of the kidney of *H.* fossilis. ***a***, Control group showing compact renal mass. RT, Renal tubules; 10 × 10 (H&E stain). ***b***, Experimental group (0.1 ml: 102 h after treatment) showing disintegrated renal tubules and ruptured blood vessel. PS, Proximal segment; 10 × 20 (H&E stain). ***c***, Experimental group (0.2 ml: 3 h after treatment) showing large space between tubules and tissue, shruk glomerulus with large Bowman's space. EL, Enlarged lumen; G, Glomerulus; 10 × 20 (H&E stain). ***d***, Experimental group (0.2 ml: 6 h day after treatment) showing disintegrated renal tubules; 10 × 20 (H&E stain). ***e***, Section of the Corpuscles of Stannius of *H. fossilis* of experimental group (0.2 ml: 15 days after treatment) showing granular cytoplasm. GC, Granular cytoplasm; 10 × 45 (H&E stain). ***f***, Section of kidney of *H. fossilis* of experimental group (0.2 ml: 10 days after treatment) showing broken blood capillary and blood entering into the tissue; 10 × 45 (H&E stain).

cells (lymphoid tissues) show necrosis and many cells show apoptosis (Figure 3).

The Corpuscles of Stannius (CS) also show same effects compared to the normal (Figure 3 *e*). In 20-day-treated kidney, CS and septae were absent. Cells were hypertrophied. Nucleus was normal and cytoplasm was granular. As CS cells are known to control the calcium metabolism in fish, it can be presumed that due to the effect on CS,

Ca metabolism of the fish is affected. However, this requires further investigation, as there is no report on the effect of microcystin on the CS of fish.

Histological changes were observed in the gonads due to toxicity of microcystin. In the testis and ovary, it was observed that compactness of the tissue was disturbed. The spermatogonia cells show necrosis. Blood was found spilling into the testis tissue (Figure 4). Not much variation could be observed in the ovary. However, the developing oocytes had less yolk, follicle cells were deformed and necrosis was also observed at some places (Figure 4 *d*, *f*). In 24 h-treated ovary, deformed tissue was observed (Figure 4 *e*). Thus we may conclude that the microcystin affects the physiology of reproduction.

The effect of microcystin injection on the concentration of bilirubin in the blood is shown in Figure 5 *a*. In both the doses (i.e. 0.1 and 0.2 ml), the concentration of bilirubin increases up to 102 h. In control, the concentration of bilirubin was 0.57 mg/dl, which increased in the treated fish in 102 h and reached up to 1.87 mg/dl in 0.1 ml dose and 2.82 mg/dl in 0.2 ml dose.

Bilirubin is the main bile pigment that is formed from the breakdown of heme in red blood cells. When *Microcystis* is injected into the fish hepatocyte necrosis occurs.

Liver damage indicated by elevated serum aspartate aminotransferase and alanine aminotransferase activity could reduce hepatic function, which may be associated with an increase in serum bile acid and bilirubin concentration[28]. Increase in bilirubin concentration was reported by Carbis *et al.*[29] in *C. carpio* due to microcystin intoxication.

In the present study also bilirubin concentration increases after 1 h of intraperitoneal injection in *H. fossilis*. Rabergh *et al.*[30] and Young *et al.*[31] have reported elevated bilirubin concentration in treated fishes.

The concentration of cholesterol in the blood of control fish was 341.3 mg/dl. It increased in the treated fish gradually and reached up to 534 mg/dl in 0.1 ml dose and 492 mg/dl in 0.2 ml dose in treated fish in 102 h after treatment (Figure 5 *b*).

The concentration of triglycerides in the blood of *H. fossilis* was 127.6 mg/dl in the control fish. It increased in 1 h and further increased gradually. However, it again decreased in the 102 h stage in both 0.1 and 0.2 ml dose experiment. Maximum concentration of triglycerides in the blood was 172 mg/dl in 0.1 ml dose and 223 mg/dl in 0.2 ml dose in treated fish. However, after 102 h of treatment, the concentration was 147 mg/dl in 0.1 ml dose and 161 mg/dl in 0.2 ml dose in treated fish (Figure 5 *c*).

In the present study also an increased level of cholesterol in the blood was observed. Increase in cholesterol and triglycerides in the blood may be due to structural damage of the liver and kidney. There is no report on cholesterol concentration in fish due to toxicity of microcystin.

Aspartate aminotransferase (AST) is found in the blood and in certain body tissues, especially the heart, liver; pancreas and muscle tissues.

Enzyme activity increases in the blood of *H. fossilis* compared to the control after intraperitoneal injection of microcystin. The concentration of AST in the blood of control fish is 157.6 (μ/l). In both concentrations (i.e. 0.1 and 0.2 ml), the enzyme activity (μ/l) of AST increases after 1 h of treatment compared to the control. Maximum activity was observed after 24 h of treatment. The value of enzymatic activity was highest compared to control, 102 h after treatment (Figure 5 *d*).

The effect of microcystin toxicity in *H. fossilis* on blood alanine aminotransferase (ALT) is shown in Figure 5 *e*. The concentration of AST in control fish blood is 59.6 (μ/l). In both concentrations (0.1 and 0.2 ml dose), the enzyme activity (μ/l) of ALT increases after 1 h of treatment compared to the control *H. fossilis*. Maximum activity was observed after 24 h of treatment; however, it starts decreasing, but always remains higher than the control blood. The increase in ALT and AST clearly shows that microcystin toxicity causes damage in the liver and kidney. Increased activity of AST and ALT in carp, *C. carpio* gavaged with single and multiple doses of microcystins has been reported by Carbis *et al.*[29]. Significant increase in ALT, AST and lactate dehydrogenase (LDH) was reported by Vajcova *et al.*[24] in silver carp, *Hypophthalmichthys molitrix* after intraperitoneal application of pure microcystin. Rabergh *et al.*[11] reported that blood plasma enzymes (ALT, AST and LDH) increase two hours after intraperitoneal injection of toxin as a consequence of hepatocyte necrosis. Kopp and Hetesa[32] have reported an increase in ALT and AST in the juvenile carp (*C. carpio*) on exposure of fish to different natural populations of cyanobacterial water blooms. Navratil *et al.*[33] have reported that the activities of ALT, AST and LDH increased after all forms of administration. The rate of increase depended on the route of administration, character of the material and the amount of toxin. Li *et al.*[34] have reported that ALT and AST activities increased significantly compared to control levels. Activities of γ-glutamyltransferase, alkaline phosphatase and LDH remained unchanged in subchronic oral toxicity of microcystin in common carp.

The activity of alkaline phosphatase in the control and treated *H. fossilis* blood is shown in the Figure 5 *f*. Concentration of alkaline phosphatase in control fish blood is 64 (μ/l). Activity of alkaline phosphatase increases after 1 h of treatment compared to the control. Maximum concentration, i.e. 118 (μ/l) in 0.1 ml dose and 160 (μ/l) in 0.2 ml dose was observed 24 h after treatment. It starts decreasing after 72 h of treatments, but always remain higher than the control blood.

Li *et al.*[34] have reported a significant increase in the activity of ALT and AST compared to the control levels, but γ-glutamyltransferase, alkaline phosphatase and LDH acti-

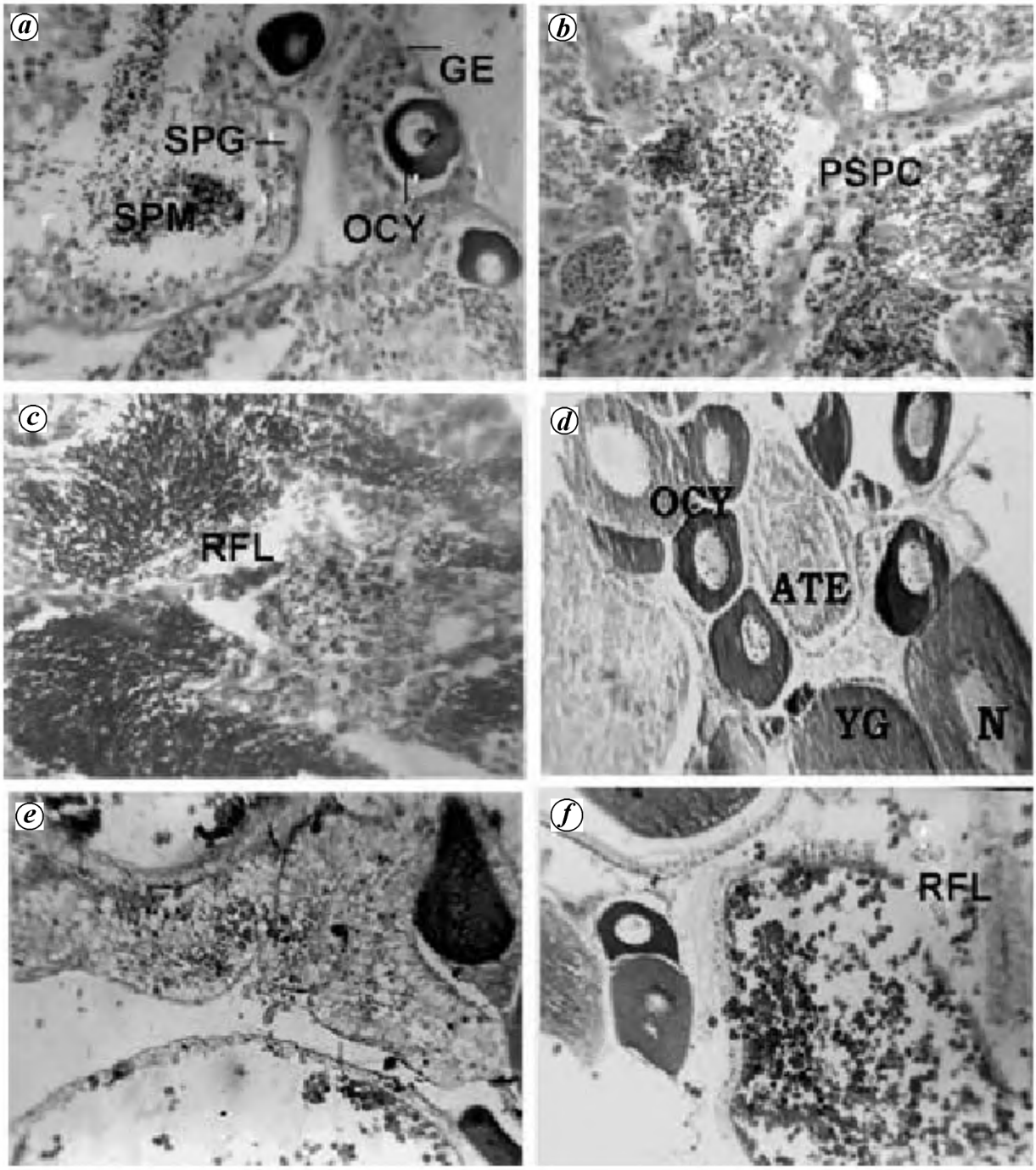

Figure 4. ***a***, Section of ovotestis of *H. fossilis* of control group showing developing oocyte from germinal epithelium. Follicle filled with sperms, large spermatogonia, primary and secondary spermatocyte. Presence of ovotestis is an exceptional case. GE, Germinal epithelium; SPG, Spermatogonia; OCY, Oocyte; SPM, Sperm; 10 × 20 (H&E. stain). ***b***, ***c***, Section of testis of *H. fossilis*. ***b***, Experimental group (0.1 ml: 10 day treatment) showing spermatogonia and primary and secondary spermatocytes with condensed chromatin material. PSPC, Primary spermatocyte; 10 × 45 (H&E stain). ***c***, Experimental group (0.2 ml: 5 days after treatment) showing ruptured follicle cells and its lining. RFL, Ruptured follicle lining; 10 × 45 (H&E stain). ***d–f***, Section of the ovary of *H. fossilis*. ***d***, Control group showing mature oocytes, nucleus, and nucleolus in the oocyte. Oocyte filled with yolk granules. YG, Yolk granules; 10 × 20 (H&E stain). ***e***, Experimental group (0.2 ml: 24 h after treatment) showing enlarged view of deformed tissue; 10 × 20 (H&E stain). ***f***, Experimental group (0.2 ml: 102 h after treatment) showing disturbed compactness of oocyte and ruptured follicle wall; 10 × 20 (H&E stain).

vities remained unchanged in subchronic oral toxicity of the microcystin experiment in common carp.

Histological observations show that the liver, gill and kidney tissues are damaged due to toxicity of microcystin and an increase in the activity of alkaline phosphatase is expected.

Variation in haemoglobin percentage in normal and treated fish is shown in Figure 6 *a* and *b*. Haemoglobin

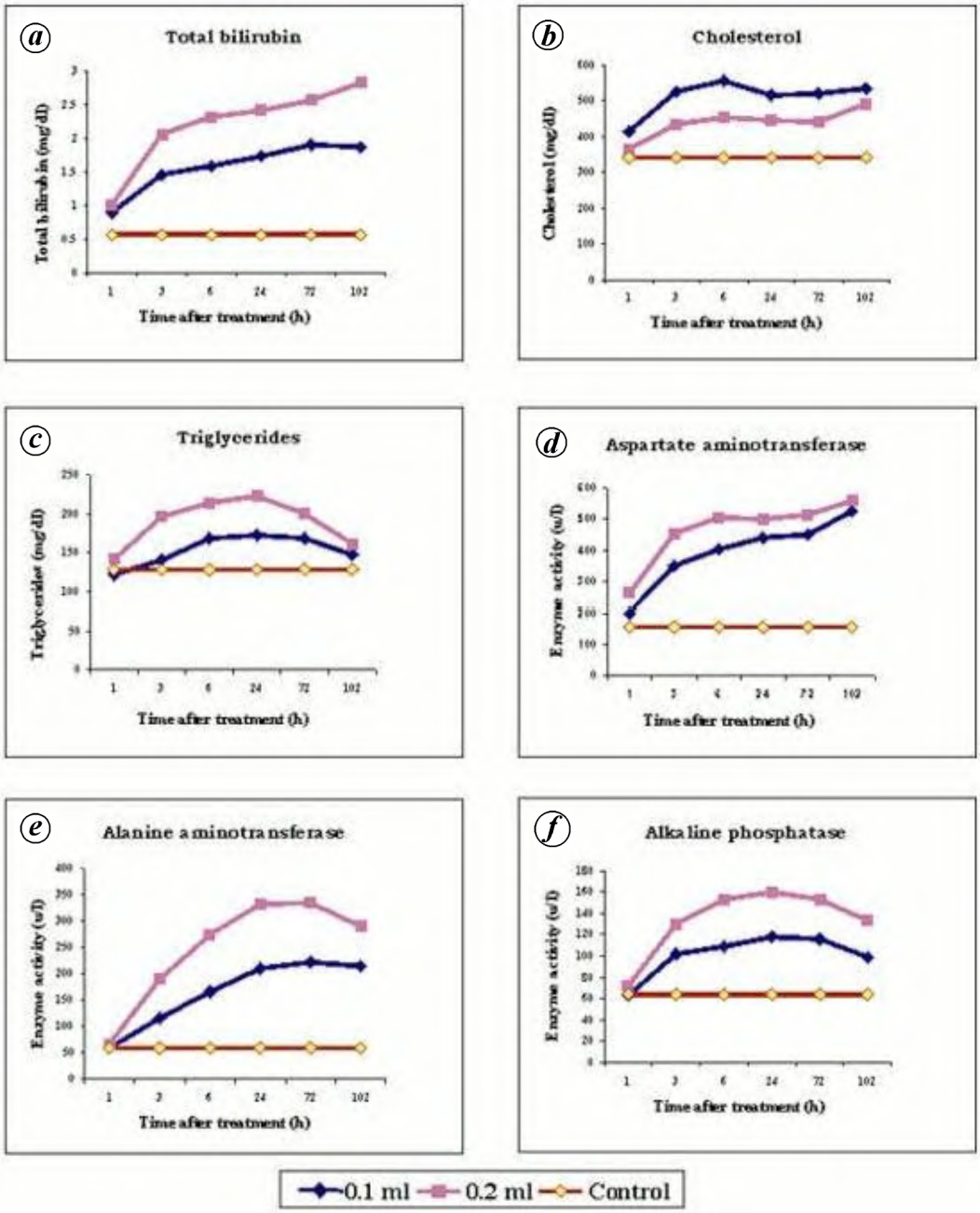

Figure 5. Total bilirubin concentration (*a*), cholesterol (*b*), Triglycerides (*c*), aspartate aminotransferase (*d*), alanine aminotransferase (*e*), and alkaline phosphatase (*f*) in blood serum of control and treated fish, *H. fossilis*.

percentage decreases after 1 h of treatment in both the doses (i.e. 0.1 and 0.2 ml). Haemoglobin percentage increases after 10 days of treatment and thereafter it becomes normal after 25 days. This shows that after 10 days the toxic effect of microcystin decreases and new haemoglobin is synthesized.

Variation in the total RBC in the blood of normal and treated fish is depicted in Figure 6 *c* and *d*. One hour after treatment, the total RBC count was high compared to the normal fish. The RBC count decreases after 6 h and up to 102 h it is lower than the control limit. After 5 days of treatment, again the total count starts increasing gradually up to 25 days and reaches the value of control fish blood. No alteration in the shape of RBC was observed in the treated fish blood.

Variation in mean corpuscular haemoglobin (MHC) in normal and treated fish is shown in Figure 6 *e* and *f*. One hour after treatment, MCH decreases as compared to the control fish blood. It increases within the hours of treatment. After 102 h of treatment, MCH values reaches near

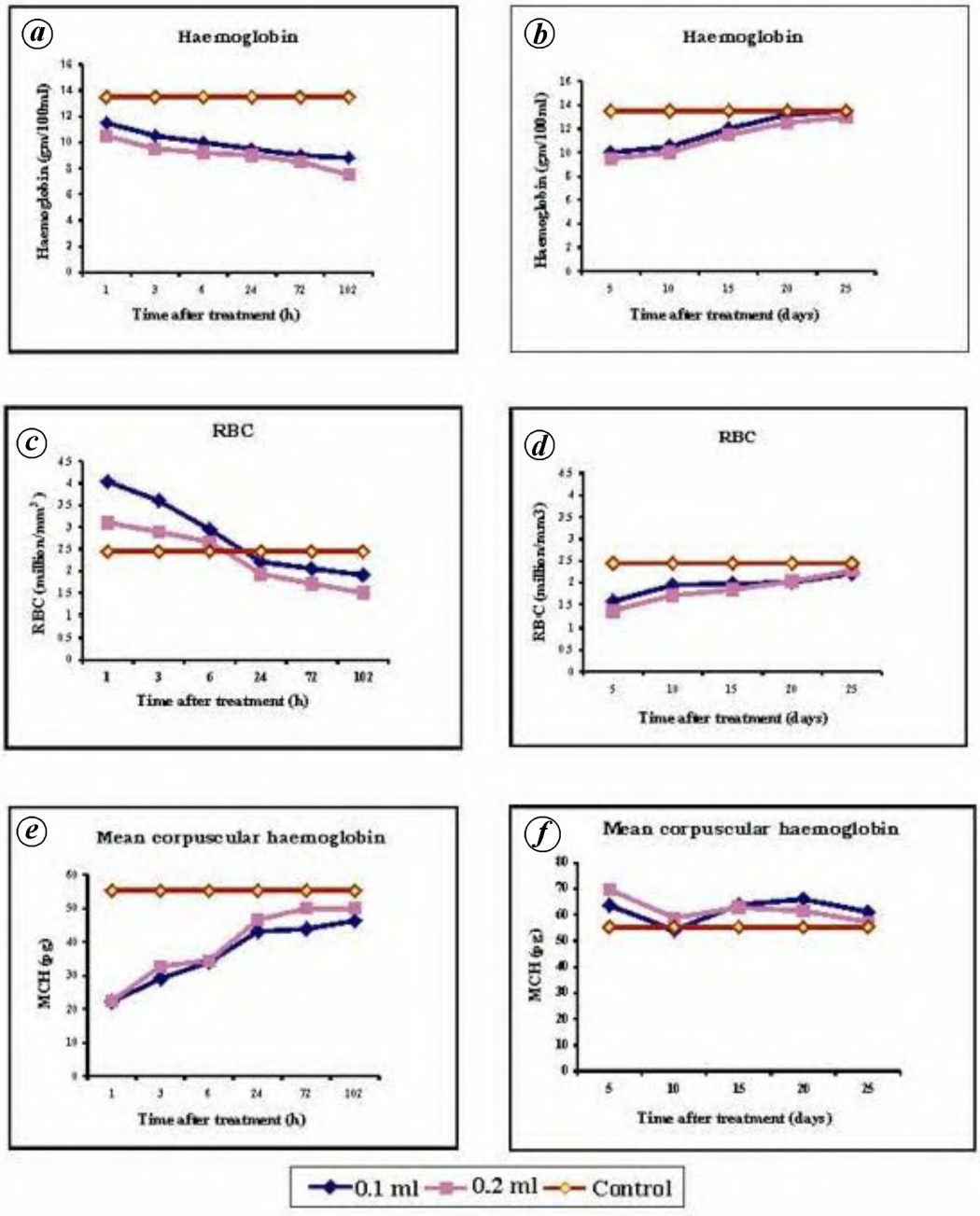

Figure 6. Haemoglobin percentage (***a***, ***b***), RBC (***c***, ***d***) and mean corpuscular haemoglobin (***e***, ***f***) in blood serum of control and treated fish *H. fossilis* (hours and days).

that of the control count. In the long-term treatment experiment, the value of MCH shows oscillations near the control value.

The effects of microcystin treatment on the haematological indices in fishes have been studied by only a few workers. No hemolysis or shape alteration was observed[35] in erythrocytes following treatment with the toxin produced by *Microcystis* in concentration up to 90 µM. In some earlier studies, toxins from *Microcystis* have been reported to cause hemagglutination and induce morphological alterations in different cell types[36,37]. In the present study no morphological change or hemagglutination was observed in the blood of *H. fossilis* after treatment with the *Microcystis* toxin. Some workers have reported the effects of microcystin on total RBC count, haemoglobin percentage and MCM concentration[32,33,38].

In the present study, a decrease in haemoglobin percentage was observed after 1 h treatment, which started increasing after 10 days of treatment and up to 25 days, after which it becomes normal. The total RBC count was

high compared to the normal fish, which again starts decreasing after 6 h and up to 102 h it becomes lower than the control. After 5 days of treatment it again starts increasing gradually up to 25 days, after which it touches the limit of control fish blood.

Navratil *et al.*[33] have reported significant changes in RBC, PCV, haemoglobin, MCV and MCH after application of the biomass of blue-green algae. Vajcova *et al.*[24] have reported a significant decrease of both haematocrit and hemoglobin concentration in silver carp (*H. molitrix*).

From these observations it can be concluded that the toxic effects due to treatment of *Microcystis* after 10 to 15 days become normal, as most of the toxin is excreted from the blood by the kidney or is metabolized into other less toxic forms.

Recent researches show that the microcystins mediate their toxicity by uptake into hepatocytes via a carrier-mediated transport system; followed by the inhibition of serine protein phosphatase 1 and 2A. The protein phosphorylation imbalance causes disruption of the liver cytoskeleton, which leads to massive hepatic haemorrhage that can cause death[39–41]. Entry of toxin into the hepatocytes of the liver and other targetted tissues is accomplished by the broad specificity anion transport bile acid carries[42].

Exposure to *Microcystis* extract resulted in increased lipid peroxidation inductive of oxidative stress in primary hepatocytes[43]. Increase in the levels of reactive oxygen species (ROS) preceded the onset of mitochondrial permeability transition and initiation of apoptosis in hepatocytes exposed to 1 μm microcystin[44]. ROS (determined by changes in H_2O_2 and O_2 concentrations) preceded the MCLR-induced collapse of the cytoskeleton and subsequent release of LDH in the hepatocytes exposed to 1 μM MCLR in the medium[45]. Addition of a superoxide dismutase mimic prevented the collapse and LDH release presumable by inactivation of ROS[46].

Apoptosis was induced in rat and salmon hepatocytes exposed to 1 μM MCLR[47]. Mice injected with sublethal toxin and killed after 7 days exhibited high levels of apoptic cells in the centrilobular and perinecrotic regions, some of which correlated with positive immunostaining for MCLR. The authors suggested a role for MCLR-induced apoptosis; however, the fact that no MCLR was found in the necrotic regions suggested a role for damage due to ischaemia/hypoxia[10].

In the present work apoptotic cells were formed in the liver, kidney, gills and testis, which were usually observed after 10 days of treatment. Guzman and Solter[48] have reported that high-dose rats showed apoptosis in the centrilobular areas, with fewer apoptotic cells visible in the medium-dose rats. Apoptotic cells were a rare occurrence in the low-dose rats.

In conclusion, *Microcystis* produce a toxin microcystin, which is of toxicological importance for humans and livestock. It is important to create awareness among the public, as *Microcystis*-producing blooms are becoming a serious global public issue. A modern water-treatment plant using pre- and intermediate ozonation steps in conjunction with on-line O_3 measurements and different filtration steps, and regular monitoring of TOC/DOC and cyanobacterial cell densities in raw water, should be able to provide safe drinking water.

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