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HISTOPATHOLOGICAL INVESTIGATIONS ON THE EFFECT OF VITAMIN C ON SODIUM FLUORIDE EXPOSED-FRESHWATER AMUR CARP, *CYPRINUS CARPIO HAEMATOPTERUS*

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ABSTRACT: The aims of the present study were to (i) examine the histopathological alterations induced by sodium fluoride exposure in the various tissues of Amur carp (*Cyprinus carpio haematopterus*) and (ii) to evaluate the ameliorative effect of dietary supplementation with vitamin C on NaF-induced toxicity in Amur carp. The experimental design involved treatment for 90 days of 150 fish divided into 5 groups of 30 which were housed in triplicate with 10 fish per tank (i) group 1 (G_1, control group); (ii) group 2 (G_2, exposure to 100 ppm NaF); (iii) group 3 (G_3, exposure to 100 ppm vitamin C supplemented diet); (iv) group 4 (G_4, exposure to both 100 ppm NaF and 100 ppm vitamin C supplemented diet), and (v) group 5 (G_5, exposure to 100 ppm NaF for the first 60 days of the 90 day treatment period and 200 ppm vitamin C supplemented diet for the last 30 days of the 90 day treatment period). Representative tissue samples of the gills, liver, spleen, intestine, and kidney were collected after every 15 day interval for histopathological examination. In groups G_1 and G_3, no histopathological changes were observed in any of the tissues examined throughout the experimental period. G_2 showed more intense microscopic lesions compared to G_4. In G_5, the severity of the histological lesions was similar to group G_2 from the 15th DPT (day post treatment) to the 60th DPT (the end of the NaF exposure) whereas from the 75th to the 90th DPT the severity of the lesions was not increased. The study concludes that sodium fluoride causes diverse adverse effects on the tissues of Amur carp exposed to 100 ppm NaF and that supplementation with 100 ppm vitamin C can be recommended in aquaculture systems to prevent the adverse effects of NaF exposure at 100 ppm. Treatment with 200 ppm vitamin C is not able to not reverse the damage already caused by 100 ppm NaF.

Keywords: Amur carp; Pathology; Sodium fluoride; Vitamin C

INTRODUCTION

The irrational release of chemicals into the water resources has potentially increased the elemental load in water, and deleteriously affected the aquatic life. The fluoride ion (F) is one of the major elemental pollutants affecting a huge number of the aquatic and terrestrial population. All over the world, around 25 nations are facing the problem of a high F content in water, above the permissible limit of 1.0–1.5 mg/L.[1] In India, most of the groundwater, in 23 out of the 37 states and union territories, is contaminated with varying amounts of F.[2] In rural areas, such fluoridated water has also been used in fish culture. F interferes with various metabolic pathways and activities[3] and induces behavioral,[4] physiological,[5] and pathological changes in fish.[6] Studies conducted on aquatic invertebrates like *Artema*, *Daphnia*, *Hydropsyche,* and *Penaeus* reveal that F affects their survival, growth, behaviour, and reproduction as well.[7]

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As an indicator of exposure to contaminants, histopathology represents a useful tool to assess the degree of pollution, particularly for sub-lethal and chronic effects.[8] The exposure to toxicants is likely to induce a number of lesions in different organs of the exposed fish, and the gills, kidney, liver, and muscle are suitable organs for histopathological examination in order to determine the effect of pollution.[9] Keeping the above facts in mind, the aims of the present study were: (i) to examine the histopathological alterations induced by sodium fluoride exposure in the various tissues of Amur carp (*Cyprinus carpio haematopterus*) and (ii) to evaluate the ameliorative effect of dietary supplementation with vitamin C on NaF-induced toxicity in Amur carp.

MATERIALS AND METHODS

Experimental fish and setup: The experiment was conducted for a period of 90 days post treatment (DPT) in the fibre-reinforced plastic (FRP) tanks (785 L capacity) in the Wet Lab of the College of Fisheries, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India. For the study, we used freshwater Amur carp (*Cyprinus carpio haematopterus*) because this species is commonly cultured in aquaculture systems in India and has 18% more growth in comparison to the other major carps. Another reason is that this species has a wide acceptability as a food which increases the likelihood that it will be eaten and result in the consumers being affected by the contaminants present in soil and water. A total of 150 apparently healthy Amur carp (average weight 170±15 g) were sourced from the Instructional Fish Farm of the College of Fisheries, Pantnagar. The fish were acclimatized for a period of 10 days prior to the start of experiment. The F content in the groundwater was estimated to be 0.05 ppm. The water was partly exchanged after every 15 days and the F concentration in the experimental tanks was monitored at every 2 days using a spectrophotometer.[10,11]

Experimental groups: A total of 150 fish were randomly divided into five groups of 30. Each group was set in triplicate with 10 fish in each tank.

(i) Group 1 (G_1): The fish were kept in ground water for 90 days.

(ii) Group 2 (G_2): The fish were exposed to 100 ppm NaF for 90 days.

(iii) Group 3 (G_3): The fish were supplemented with 100 ppm vitamin C in their diet for 90 days.

(iv) Group 4 (G_4): The fish were simultaneously exposed to 100 ppm NaF and supplemented with 100 ppm vitamin C in their diet for 90 days.

(v) Group 5 (G_5): The fish were exposed to 100 ppm NaF for 60 days and then supplemented with 200 ppm vitamin C in their diet for the last 30 days of the 90 day treatment period.

The feeding was done at the rate of 5 percent of the total body weight of the fish per day. The daily dose was split in two with half being given twice daily.

Chemicals: The sodium fluoride was purchased from HiMedia Laboratories, Mumbai, India.

Sampling schedule: Three fish, chosen randomly from each group, were slaughtered in a humane way, and the tissues were collected on post treatment days

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(PTD) 0, 15, 30, 45, 60, 75, and 90. The fish were taken out from the experimental tanks using a hand net and were anesthetized using clove oil. A postmortem examination of the fish was done and representative tissue samples were collected from the gills, liver, spleen, intestine, and kidney from all the groups. The tissues were collected in 10% neutral buffered formalin.[12] The preserved tissue samples were processed for histology as described elsewhere[13] and then the histopathological changes were examined under a light microscope and the images of the lesions were captured by a camera mounted on the microscope.The lesions were graded and scored as mild, moderate, and severe. The mean lesion score of the 3 fish from each group at each time interval was calculated.

RESULTS

The histopathological lesion score in the various organs of the different experimental groups at the different DPT is depicted in Tables 1–5. No histopathological changes were recorded for any of the organs examined in groups G_1 and G_3 throughout the study period.

Table 1. Mean histopathological lesion score for the gill of the different experimental groups at different days post treatment

Days post treatment (DPT)	Histopathological lesion score for the gill* Experimental group†				
	G_1	G_2	G_3	G_4	G_5
0	–	–	–	–	–
15	–	++	–	+	++
30	–	+++	–	+	+++
45	–	+++	–	+	+++
60	–	+++	–	+	+++
75	–	+++	–	+	++
90	–	+++	–	+	++

*Histopathological lesion score: –: no lesion; +: mild; ++: moderate; +++: severe.

†G_1: The fish were kept in ground water for 90 days.

G_2: The fish were exposed to 100 ppm NaF for 90 days.

G_3: The fish were supplemented with 100 ppm vitamin C in their diet for 90 days.

G_4: The fish were simultaneously exposed to 100 ppm NaF and supplemented with 100 ppm vitamin C in their diet for 90 days.

G_5: The fish were exposed to 100 ppm NaF for 60 days and then supplemented with 200 ppm vitamin C in their diet for the last 30 days of the 90 day treatment period.

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Table 2. Mean histopathological lesion score for the liver of the different experimental groups at different days post treatment

Days post treatment (DPT)	Histopathological lesion score for the liver* Experimental group†				
	G_1	G_2	G_3	G_4	G_5
0	–	–	–	–	–
15	–	++	–	+	++
30	–	+++	–	+	+++
45	–	+++	–	+	+++
60	–	+++	–	+	+++
75	–	+++	–	+	++
90	–	+++	–	+	++

*Histopathological lesion score: –: no lesion; +: mild; ++: moderate; +++: severe.
†G_1: The fish were kept in ground water for 90 days.
G_2: The fish were exposed to 100 ppm NaF for 90 days.
G_3: The fish were supplemented with 100 ppm vitamin C in their diet for 90 days.
G_4: The fish were simultaneously exposed to 100 ppm NaF and supplemented with 100 ppm vitamin C in their diet for 90 days.
G_5: The fish were exposed to 100 ppm NaF for 60 days and then supplemented with 200 ppm vitamin C in their diet for the last 30 days of the 90 day treatment period.

Table 3. Mean histopathological lesion score for the spleen of the different experimental groups at different days post treatment

Days post treatment (DPT)	Histopathological lesion score for the spleen* Experimental group†				
	G_1	G_2	G_3	G_4	G_5
0	–	–	–	–	–
15	–	–	–	–	–
30	–	–	–	–	–
45	–	–	–	–	–
60	–	–	–	–	–
75	–	+	–	–	–
90	–	+	–	–	–

*Histopathological lesion score: –: no lesion; +: mild; ++: moderate; +++: severe.
†G_1: The fish were kept in ground water for 90 days.
G_2: The fish were exposed to 100 ppm NaF for 90 days.
G_3: The fish were supplemented with 100 ppm vitamin C in their diet for 90 days.
G_4: The fish were simultaneously exposed to 100 ppm NaF and supplemented with 100 ppm vitamin C in their diet for 90 days.
G_5: The fish were exposed to 100 ppm NaF for 60 days and then supplemented with 200 ppm vitamin C in their diet for the last 30 days of the 90 day treatment period.

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Table 4. Mean histopathological lesion score for the intestine of the different experimental groups at different days post treatment

Days post treatment (DPT)	Histopathological lesion score for the intestine*				
	Experimental group†				
	G_1	G_2	G_3	G_4	G_5
0	–	–	–	–	–
15	–	++	–	+	++
30	–	+++	–	+	+++
45	–	+++	–	+	+++
60	–	+++	–	+	+++
75	–	+++	–	+	++
90	–	+++	–	+	++

*Histopathological lesion score: –: no lesion; +: mild; ++: moderate; +++: severe.
†G_1: The fish were kept in ground water for 90 days.
G_2: The fish were exposed to 100 ppm NaF for 90 days.
G_3: The fish were supplemented with 100 ppm vitamin C in their diet for 90 days.
G_4: The fish were simultaneously exposed to 100 ppm NaF and supplemented with 100 ppm vitamin C in their diet for 90 days.
G_5: The fish were exposed to 100 ppm NaF for 60 days and then supplemented with 200 ppm vitamin C in their diet for the last 30 days of the 90 day treatment period.

Table 5. Mean histopathological lesion score for the kidney of the different experimental groups at different days post treatment

Days post treatment (DPT)	Histopathological lesion score for the kidney*				
	Experimental group†				
	G_1	G_2	G_3	G_4	G_5
0	–	–	–	–	–
15	–	++	–	+	++
30	–	+++	–	+	+++
45	–	+++	–	+	+++
60	–	+++	–	+	+++
75	–	+++	–	+	++
90	–	+++	–	+	++

*Histopathological lesion score: –: no lesion; +: mild; ++: moderate; +++: severe.
†G_1: The fish were kept in ground water for 90 days.
G_2: The fish were exposed to 100 ppm NaF for 90 days.
G_3: The fish were supplemented with 100 ppm vitamin C in their diet for 90 days.
G_4: The fish were simultaneously exposed to 100 ppm NaF and supplemented with 100 ppm vitamin C in their diet for 90 days.
G_5: The fish were exposed to 100 ppm NaF for 60 days and then supplemented with 200 ppm vitamin C in their diet for the last 30 days of the 90 day treatment period.

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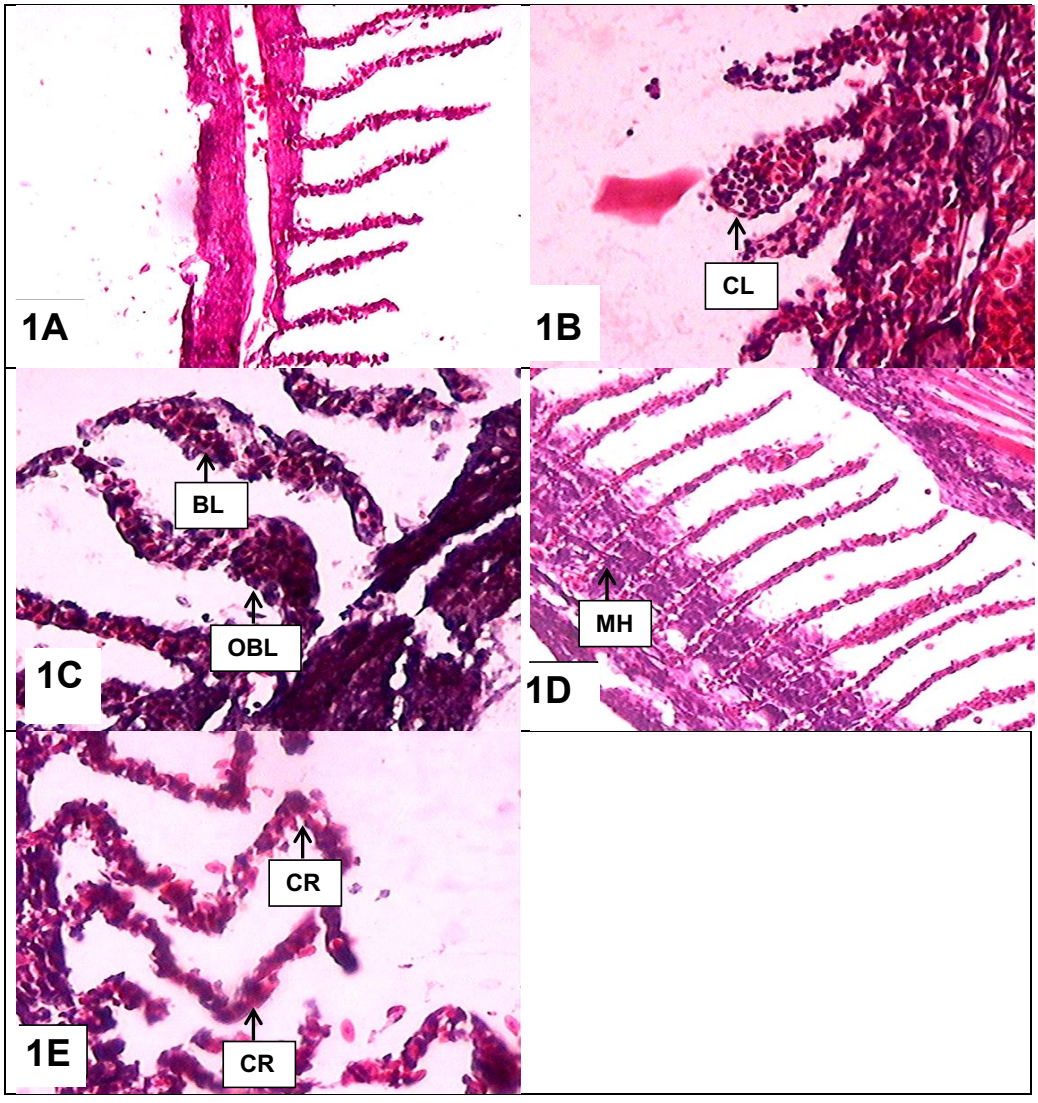
Gills: The various histopathological lesions in the gills of the G_1 (fish kept in ground water for 90 days) and G_2 (fish exposed to 100 ppm NaF for 90 days) groups at the different DPTs are depicted in Figures 1A–1E.



Figures 1A–1E. Microphotographs of gill showing 1A: no lesion (Group G_1, 0th DPT, H&E×100); 1B: clubbing (CL) of the tip of secondary gill lamellae (Group G_2, 15th DPT, H&E×200); 1C: bulging at the base (BL) and overall bulging (OB) of secondary gill lamellae (Group G_2, 30th DPT, H&E×200); 1D: mucoid hyperplasia (MH) (Group G_2, 45th DPT, H&E×100); and 1E: curling (CR) of secondary gill lamellae (Group G_2, 60th DPT, H&E×200). DPT=days post treatment.
G_1: The fish were kept in ground water for 90 days.
G_2: The fish were exposed to 100 ppm NaF for 90 days.

The histopathological studies of gills of groups G_2 and G_5, at the 15th DPT, showed overall thickening of the secondary gill lamellae, mucoid hyperplasia, blunting and clubbing of the tip of the secondary gill lamellae, congestion of the large blood vessels of gills, curling, and severe congestion of the secondary gill lamellae. At the 30th DPT, in groups G_2 and G_5, the gills manifested similar lesions with a higher

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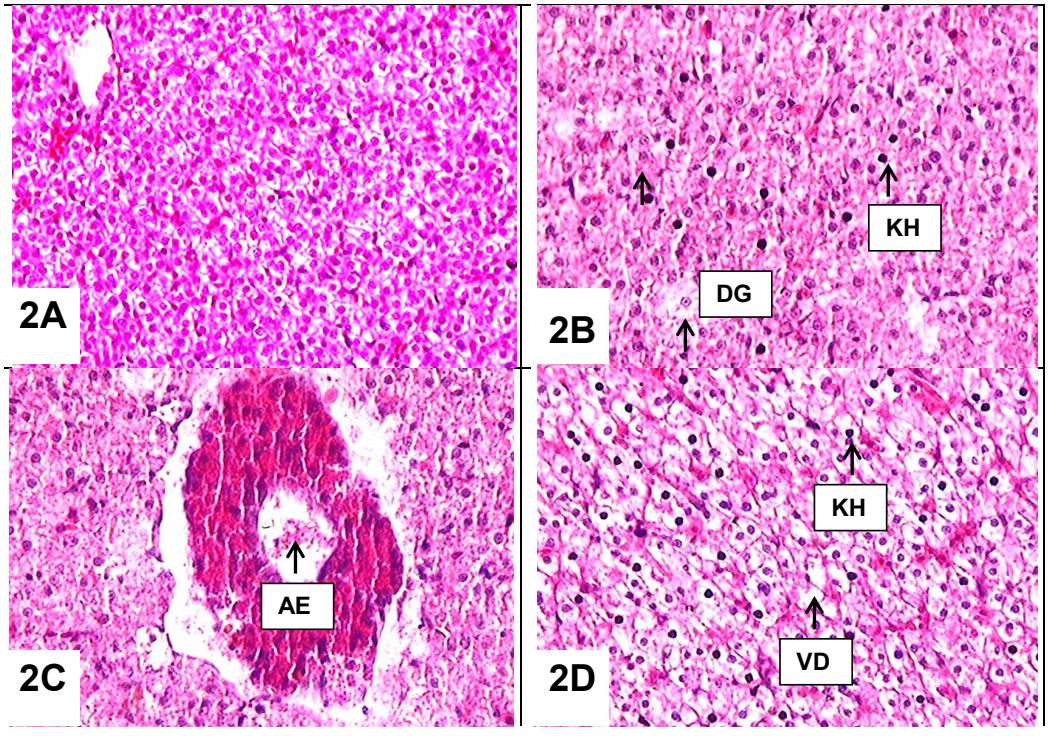
severity and many of the secondary gill lamellae showed curvature from the tip side. At the 45th DPT, the histopathological lesions included thickening and blunting of secondary gill lamellae and severe mucoid hyperplasia at many foci. There were swelling and curling of the secondary gill lamellae along with severe reduction in the size of secondary gill lamellae at the 60th DPT. At the 75th and 90th DPT, in group G_2, similar lesions were observed but of a greater severity. In group G_5, at the 75th and 90th DPT, the severity of the lesions was less than in group G_2. In group G_4, similar but moderate lesions could be recorded including moderate shortening and thickening of the secondary gill lamellae and mucoid hyperplasia.

Liver: The various histopathological lesions in the liver of the G_1 (fish kept in ground water for 90 days) and G_2 (fish exposed to 100 ppm NaF for 90 days) groups at the different DPTs are depicted in Figures 2A–2G.



Figures 2A–2D. Microphotographs of liver showing 2A: no lesion (Group G_1, 0th DPT, H&E×100); 2B: degeneration of the hepatocytes (DG) and Kupffer cell hyperplasia (KH) (Group G_2, 15th DPT, H&E×200); 2C: accumulation of erythrocytes (AE) in hepatopancreas (Group G_2, 15th DPT, H&E×200); and 2D: severe vacuolar degeneration (VD) and Kupffer cell hyperplasia (KH) (Group G_2, 45th DPT, H&E×200). DPT=days post treatment.

G_1: The fish were kept in ground water for 90 days.

G_2: The fish were exposed to 100 ppm NaF for 90 days.

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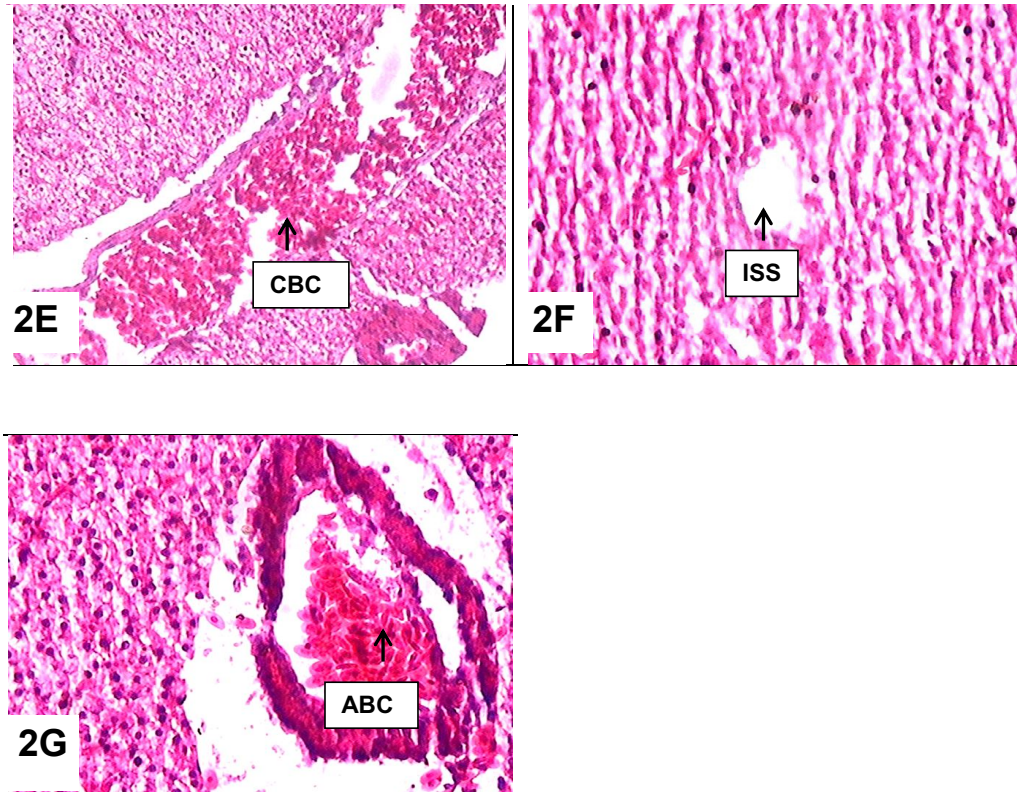

Figures 2E–2G. Microphotographs of liver showing 2E: severe clumping of blood corpuscles (CBC) in a large blood vessel (Group G_2, 45th DPT, H&E×100); 2F: shrinkage of hepatic cells leading to increased sinusoidal spaces (ISS) (Group G_2, 60th DPT, H&E×200); and 2G: accumulation of blood corpuscles (ABC) in hepatopancreas (Group G_2, 90th DPT, H&E×200). DPT=days post treatment.
G_2: The fish were exposed to 100 ppm NaF for 90 days.

Microscopically, the liver of groups G_2 and G_5, at the 15th DPT, showed vacuolar degeneration of hepatocytes leading to a massive swelling of the hepatocytes resulting in the loss of sinusoidal spaces. There were coagulative necrosis, congestion of the large blood vessels, Kupffer cell hyperplasia at many places, and severe congestion of the hepatopancreas. In groups G_2 and G_5, at the 30th and 45th DPT, the histopathological lesions were similar but more severe than at the 15th DPT, whereas at the 60th DPT there was a severe shrinkage of the hepatic cells throughout the parenchyma leading to increased sinusoidal spaces and Kupffer cell hyperplasia. In group G_2, at the 75th and 90th DPT, the same lesions were seen with more severity. In group G_5, at the 75th and 90th DPT, the severity of the lesions was less than in group G_2. In group G_4, the lesions were of moderate intensity, compared to the severe lesions in group G_2, and comprised mild Kupffer cell hyperplasia and mild degenerative changes in the hepatocytes.

Spleen: The various histopathological lesions in the spleen of the different groups at the different DPTs are depicted in Figures 3A and 3B.

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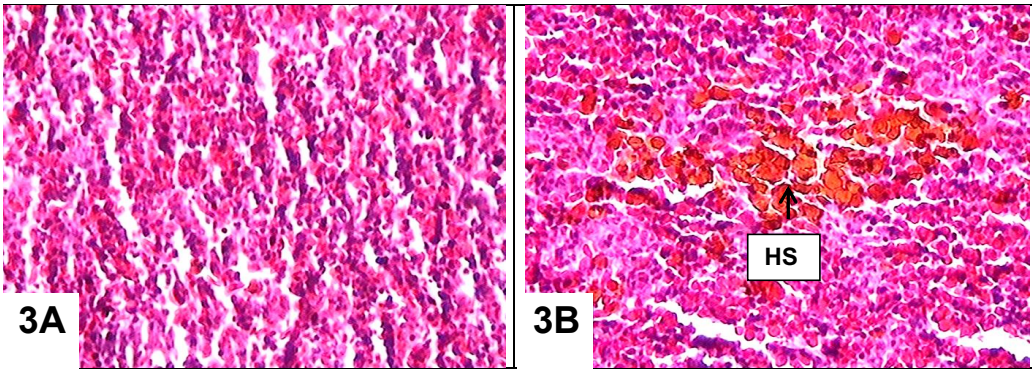
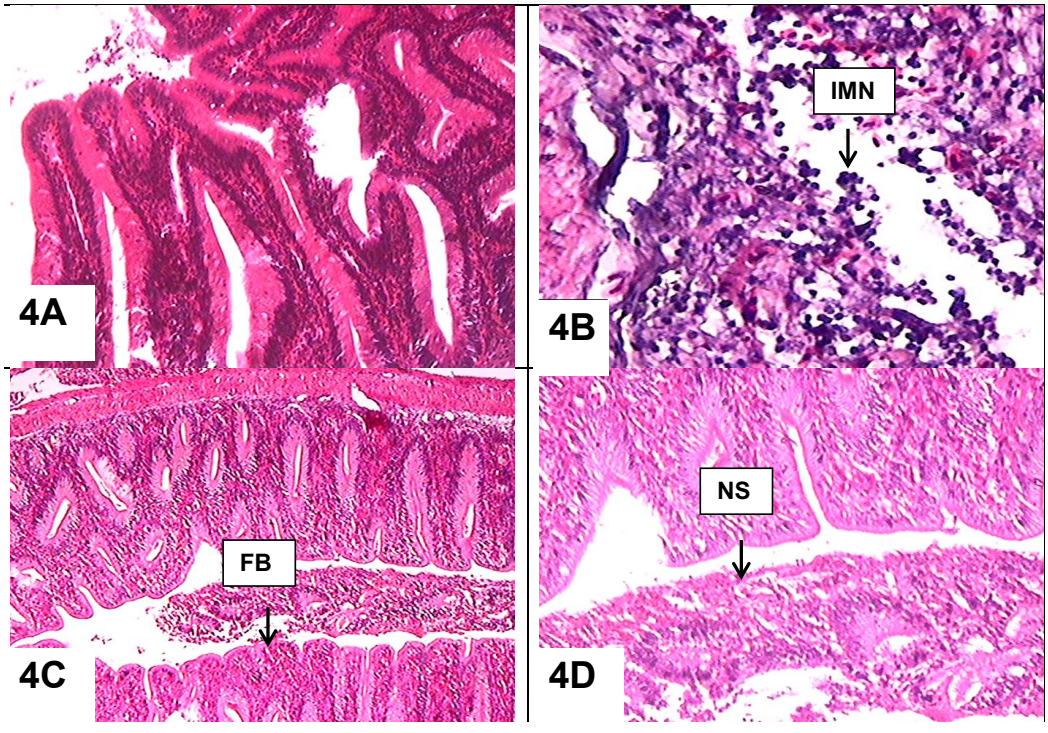


Figures 3A and 3B. Microphotograph of spleen showing 3A: no lesion (Group G_1, 0th DPT, H&E× 200); 3B hemosiderosis (HS) (Group G_2, 75th DPT, H&E×100). DPT=days post treatment.
G_1: The fish were kept in ground water for 90 days.
G_2: The fish were exposed to 100 ppm NaF for 90 days.

The spleen, in group G_2, from the 15th to the 60th DPT, showed no significant changes whereas at the 75th and 90th DPT, there were mild histopathological lesions with multifocal hemosiderosis at many places. No lesions were seen in groups G_1, G_3, G_4, and G_5 at any of the DPTs.

Intestine: The various histopathological lesions in the intestine of the different groups at the different DPTs are presented in Figures 4A–4G.



Figures 4A–4D. Microphotographs of intestine showing 4A: no lesion (Group G_1, 0th DPT, H&E×100); 4B: infiltration of mononuclear cells (IMN) in the mucosa (Group G_2, 30th DPT, H&E× 100); 4C: massive fusion and blunting of villi (FB) (Group G_2, 45th DPT, H&E×400); and 4D: necrosis and sloughing (NS) of villi leading to small sized villi (Group G_2, 45th DPT, H&E×100). G_1: Fish kept in ground water for 90 days. G_2: Fish exposed to 100 ppm NaF for 90 days.

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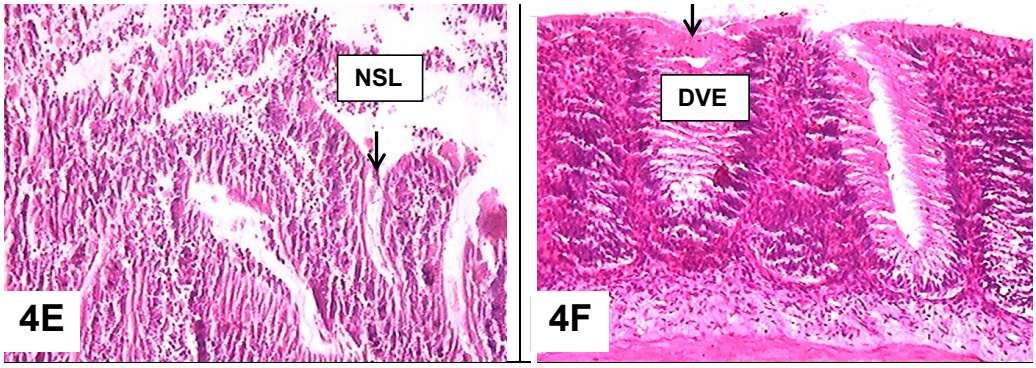
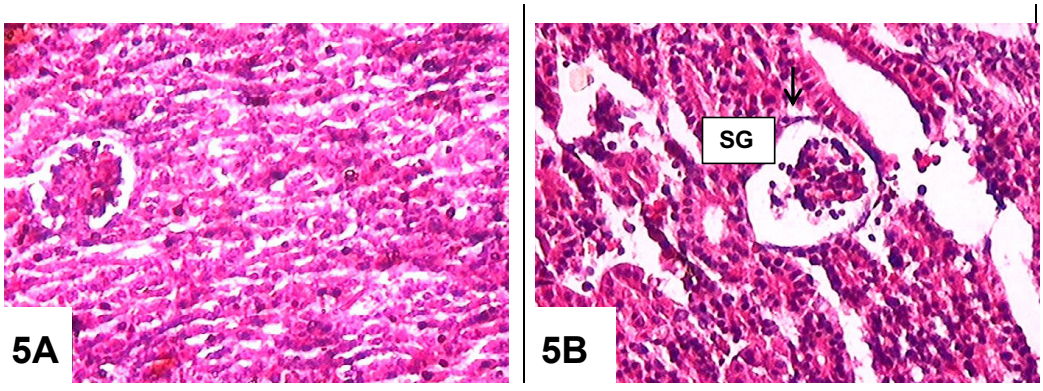


Figures 4E and 4F. Microphotograph of intestine showing 4E: necrosis and sloughing of villi (NSL) (Group G_2, 45th DPT, H&E×100); and 4F: degeneration of villous epithelium (DVE) (Group G_2, 75th DPT, H&E×100). DPT=days post treatment.
G_2: The fish were exposed to 100 ppm NaF for 90 days.

The lesions in the intestine in groups G_2 and G_5, at the 15th DPT, comprised degenerative changes in the mucosal glands. At the 30th and 45th DPT, there was necrosis, sloughing, massive fusion and blunting of villi, and infiltration of mononuclear cells at many places in the mucosa. Small sized villi due to necrosis and sloughing were also recorded. In groups G_2 and G_5, at the 60th DPT, the intestine manifested severe blunting of the villi and degeneration of villous epithelium. At the 75th and 90th DPT, in group G_2, the lesions were similar but of a severe nature. In group G_5, at the 75th and 90th DPT, the severity of the lesions was less than those in group G_2 at these DPTs. In group G_4, the lesions were moderate compared to the severe lesions in group G_2, and these involved mild fusion and blunting or thickening of the intestinal villi.

Kidney: The diverse histopathological lesions in the kidneys of the different groups at the different DPTs are shown in Figures 5A–5D.



Figures 5A and 5B. Microphotographs of kidney showing 5A: no lesion (Group G_1, 0th DPT, H&E×200); 5B: shrinkage of glomeruli (SG) (Group G_1, 45th DPT, H&E×200). DPT=days post treatment.
G_1: The fish were kept in ground water for 90 days.
G_2: The fish were exposed to 100 ppm NaF for 90 days.

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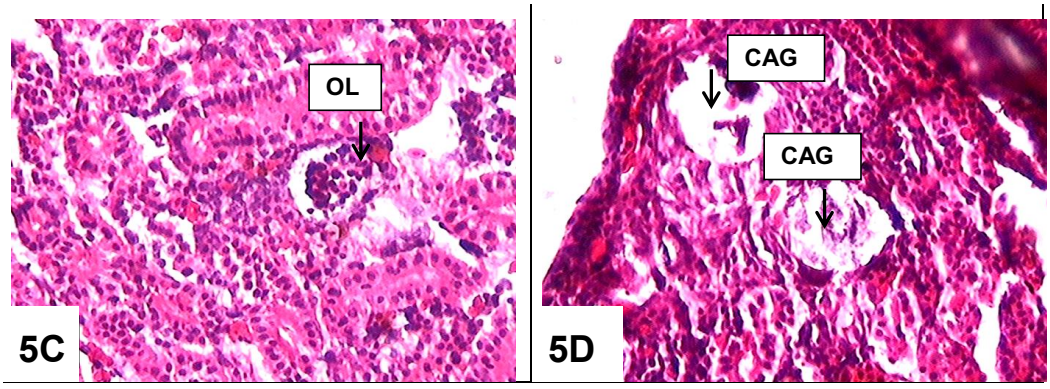

Figures 5C and 5D. Microphotographs of kidney showing 5C: swelling of the KTE cells leading to obliteration of the lumen (OL) of the tubule (Group G_2, 45th DPT, H&E×200); (d) complete absence of the glomeruli (CAG) (Group G_2, 90th DPT, H&E×200). DPT=days post treatment. G_2: The fish were exposed to 100 ppm NaF for 90 days.

The pathological lesions in the kidneys of groups G_2 and G_5, at the 15th and 30th DPT, comprised shrinkage of glomeruli, degeneration of the kidney tubular epithelial (KTE) cells, and destruction of the kidney tubules at many places. Shrinkage of glomeruli and swelling of the KTE cells leading to obliteration of the lumen of the tubule was evident in groups G_2 and G_5 at the 45th and 60th DPT. In group G_2, at the 75th DPT, similar lesions of high severity were observed whereas at the 90th DPT the lesions showed severe shrinkage of the glomeruli and at places a complete absence of glomeruli from the Bowman's space. In group G_5, at the 75th and 90th DPT, the severity of these lesions was less in comparison to group G_2 at these DPTs. In group G_4, the lesions were less severe than in group G_2, and the lesions were a mild reduction in the size of glomeruli at very few places, mild shrinkage of glomeruli, and mild degenerative changes in the KTE cells.

DISCUSSION

As the pollutants in the aquatic environment enter the fish body through the aquatic food chains, they can accumulate in the different tissues or organ systems and alter various metabolic and physiological processes. The changes induced by the pollutant at the tissue level can be assessed by histopathological markers. Hence, it can be considered to be able to reflect the overall health of the entire population in the particular ecosystem.[14] Reactive oxygen species (ROS), as a by-product of the metabolic processes, can be scavenged by many antioxidative defense components under normal conditions[15] whereas an imbalance between ROS and antioxidants creates oxidative stress.[16] F freely and rapidly migrates across the biological membranes[17]and inhibits the antioxidant enzymes, which in turn promote the accumulation of ROS[18, 19] and alter the antioxidative parameters. F induces oxidative stress which results in damage to various tissues.[20] Various types of tissue damage and structural changes caused by the F exposure have been observed and reported in the freshwater fishes *Labeorohita*, *Heteropneustes fossilis,* and *Channapunctatus*, respectively.[6,21,22] The observations of the present study are consistent with the findings in these studies.

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In general, the severity of the histopathological lesions in the examined tissues increased with the duration of F exposure in groups G_2 and G_5.

The gills are the primary organ that come in contact with and are affected by the toxicants present in water.[23] An increase in the thickness and a merger of the secondary lamellae could be a result of edematous swelling and hypertrophy of the epithelial cells. This may increase the respiratory gaseous diffusion distance between the water surrounding the gills and the blood in the blood spaces between the pillar cells of the secondary lamellae, resulting in a decrease in the efficiency of the exchange of the respiratory gases.[24] The exposure to chemicals may damage the gills and weaken the ability to maintain a proper ionic balance.[25] The gills of the F exposed-freshwater fish (*Labeorohita*) showed a swelling at the tip of the secondary gill lamellae, clubbing of lamellae, mucoid metaplasia, and lamellar hyperplasia.[6] In another freshwater fish species, *Heteropneustes fossilis*, on exposure to F, the major alterations observed during the initial stage of toxicity involved swelling and degeneration in the primary and secondary gill lamellae whereas the later changes were clubbing of the gill tips, hyperplasia, and fusion of the gill lamellae.[21] These studies indicate that F alters the normal histoarchitecture of the gills but the severity of the histological alterations depends on the duration of the F exposure. The severity of F-induced toxicity is affected by the F concentration, the duration and frequency of the exposure,[26] age,[27]and the specie of animal.[28]

The liver, is responsible for maintaining bodily metabolic homeostasis and has been considered to be the target organ for the toxic effects of fluoride.[25] The disturbance of the pro-oxidant and antioxidant balance by the generation of reactive oxygen species causes liver damage. The liver is one of the active metabolism sites that is susceptible to F-induced toxicity.[29] Degeneration of liver hepatocytes has been observed in the hepatic tissue of F-exposed *Channa punctatus*[21] and *Rasbora daniconius*[30] compared to control cells. Researchers have also been observed that F exposure caused vacuolization, presence of pyknotic nuclei, disruption, rupture, hypertrophy, and hyperplasia of liver hepatocytes in the fresh water fishes *Heteropneustes fossilis*[31] and *Odontesthes bonariensis*.[32]

In the present study, the spleen of the F exposed-fish showed an accumulation of the hemosiderin component. Hemosiderosis is due to an increased rate of erythrocyte destruction in the spleen[33] which. in the present case, is perhaps a consequence of the F exposure of the Amur carp. This in turn may result in a decreased hemoglobin content which is usually attributed in fishes to red blood cell (RBC) destruction and an irregular movement of hemoglobin from the spleen.[34] The hemosiderin deposition has also been observed in the spleen of *Odontesthes bonariensis* sampled from Lake Chasico, Buenos Aires, Argentina, whose water contains a high amount of F.[32]

The acidic pH of the stomach is due to HCl secreted from the parietal cells of stomach wall. In the presence of F ions, the HCl combines with the F ions and forms hydrofluoric acid (HF) which has highly corrosive properties and thereby causes pathogenicity in the intestine. The HF destroys the mucus secreting cells of the intestinal lining causing various abnormalities as observed in the present study. F exposed-intestinal tissue showed a degeneration of the villi with a severe necrosis in the absorptive columnar epithelial cells.[22]

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The kidney can be considered as a target organ for studying the adverse effects of F due to its bio-concentration, the kinetics of F metabolism, and the F excretion patterns.[36] F enhances lipid peroxidation (LPO) and inhibits the antioxidant enzymes in the liver and kidney. The increase in the LPO by F can be due to increased oxidative stress in the cell as a result of a depletion of the antioxidant scavenger system.[37]Microscopic changes, like disruption of the Bowman's capsule along with glomerular shrinkage, and vacuolation in the epithelial cell lining of the renal tubules, have been observed in the kidney of F exposed *Channa punctatus*[22] and *Poecilia reticulata*.[38]

Vitamin C is an important water soluble antioxidant which is reported to neutralize reactive oxygen species (ROS) and reduce the oxidative stress thereby lowering the risk of tissue damage and its dysfunctioning.[39] Antioxidant compounds can prevent the uncontrolled formation of free radicals or inhibit their reaction with biological sites. In addition, the destruction of most free radicals depends on the oxidation of endogenous antioxidants mainly by scavenging, and reducing molecules.[40] The potential of vitamin C for suppressing the oxidative stress and toxicity caused by the F exposure has also been reported by various researchers.[41- 43]

In the experimental group G_5, 200 ppm vitamin C was incorporated in the diet for the last 30 days of the 90 day treatment period after exposing the Amur carp to 100 ppm NaF for 60 days. The damage caused by the F toxicity was not reversed by the vitamin C supplementation which could be due to the dose of vitamin C or the duration of the supplementation being insufficient. As observed in the present study, F-induced toxicity is time dependent. Vitamin C can reduce or prevent the causation of damage as evident in G_4 but it was not able to reverse the damage already done. To reduce the F induced-toxicity, vitamin C can also be utilized in combination with other protective agents, like vitamin E, *Ginkgo biloba*, chlorpyrifos, and quercetin, to increase the protective effect.[42-46] On the other hand, the toxicity did not increase further after vitamin C supplementation which could be due to the scavenging property of vitamin C that protected the organs from further damage.[41]

The outcomes of the present study can be used in aquaculture systems where the water F concentration is higher than the permissible limit.[1]As synthetic vitamin C is easily available in the market, the problem of F toxicity and F accumulation can be controlled in aquaculture systems by using vitamin C and subsequently the occurrence of fluorosis can be prevented in the humans who are the ultimate consumers of the fish and other aquatic products.

CONCLUSIONS

It can be concluded from the present study that sodium fluoride, in a concentration of 100 ppm, can adversely affect the tissues of Amur carp (*Cyprinus Carpio haematopterus*) and that dietary supplementation with 100 ppm vitamin C can be recommended for use in aquaculture systems to prevent F-induced adverse effects. In addition, the findings indicate that dietary treatment with 200 ppm vitamin C is not able to reverse existing NaF induced-damage.

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