HISTOPATHOLOGICAL AND ULTRAMICROSCOPIC CHANGES INDUCED BY FLUORIDE IN SOFT TISSUE ORGANS OF THE AIR-BREATHING TELEOST, *CHANNA PUNCTATUS* (BLOCH)

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SUMMARY: The air-breathing teleost, *Channa punctatus* (Bloch), was exposed to sodium fluoride (NaF) at a sublethal dose of 40 mg/L for a period of 45 days under laboratory conditions to study the histopathological and ultramicroscopical alterations in the stomach, intestine, liver, and kidney. Severe vacuolation in the gastric epithelium and disruption in the tubular gastric glands of the stomach occurred. Disarrangement of mucosal folds, degeneration of epithelial cells, and loss of microridges with vigorous mucus secretion were also the prominent features in the stomach. In the intestine, the villi showed degeneration with severe necrosis in absorptive columnar epithelial cells and fusion of the cells at the basal region. The absorptive luminal surface area was reduced from disruption of primary and secondary mucosal folds. The absorptive columnar epithelial cells lost much of their identity, forming an even cell sheet resulting into impairment of absorption of essential nutrients. The centrolobular area of the liver exhibited focal necrosis. The cytoplasm of the hepatocytes degenerated, nuclei showed reduction in volume and pyknotic condition with syncytial appearance. Zymogen granules became scattered in the hepatopancreatic acinar cells, resulting into degranulation and vacuolation of the cells. In the kidney, the most pronounced histopathological changes were disruption of Bowman's capsule and deterioration in the epithelial cell lining of renal tubules, particularly in the proximal tubules.

Keywords: Air-breathing teleost; *Channa punctatus* (Bloch); F-induced toxicity; Fish intestine and stomach; Fish kidney and liver; Scanning electron microscopy; Soft-tissue organs; Teleost histopathology.

INTRODUCTION

Various forms of inorganic fluorides, including sodium fluoride (NaF), have found various uses in agriculture, industry, and commerce. A significant amount of environmental fluoride (F) input comes from industrial and domestic discharge, run off from indiscriminate use of pesticides and fertilizers in agriculture, solubilization of F-bearing rocks from precipitation, and through exposure to groundwater sources. The high cell-membrane penetrating power, bioaccumulation, and non-biodegradable property of F cause it to have a major impact on ecotoxicology.[1] In particular, aquatic organisms are often exposed to high concentration of F resulting in bioaccumulation.[2,3]

Among humans, especially in India, fluorosis from drinking water contamination is a common major health problem only partially correctable by dietary improvement.[4] Much research has been done on F-induced skeletal malformation, growth, biochemical disturbances, and pathological changes in tissues of human and other mammals, and some kinds of fish.[5-13] Genotoxicity study of F has also been conducted on fish.[14] However, histopathological and ultrastructural alterations in fish caused by F is relatively scanty. The present study was conducted to determine the toxicity of F at cellular and subcellular levels in a

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benthic teleostean fish, *Channa punctatus* (Bloch), particularly in the soft tissue organs of the stomach, intestine, liver, and kidney.

MATERIALS AND METHODS

Experimental design: A total of twenty adult *Channa punctatus* having an average length of about 22.0±2.0 cm and weighing about 150.0±5.0 g were collected from a local pond and acclimatized under laboratory conditions for 10 days. During acclimatization the fish were fed with live *Tubifex* sp. After acclimatization, the fish were divided into two equal groups of ten each in two aquaria of 250-L capacity. One group was exposed to a sublethal dose of 40 mg NaF/L (= 18 mg F ion/L) for 45 days, applied on every alternate day, while the other group was maintained as control. To maintain the water quality, the aquarium water was changed for every alternate day. Live *Tubifex* sp. food was added on a regular basis, and treatment with NaF was renewed accordingly.

Scanning electron microscopic fixation: After 45 days of treatment, the fish from the two aquaria were anaesthetized with tricaine methonesulphonate (MS 222). For ultrastructural study the representative regions of the alimentary canal viz., stomach and intestine were removed immediately after dissection, and the luminal surface exposed through longitudinal incision. The luminal surface of the excised tissues was spread out and pinned on the thermocol. The adhering excess mucus on the luminal surface was removed by rinsing in heparinized saline. After further rinsing in 0.1 M cacodylate buffer at pH 7.5, the tissues were infiltered with 2.5% glutaraldehyde for 24 hr fixation at 4ºC. After fixation, the tissues were removed, rinsed in buffer, trimmed into 8.0-mm squares and subjected to post-fixation in 1% OsO_4 in 0.1 M cacodylate buffer at pH 7.5 for 2 hr, dehydrated through graded acetone followed by amyl acetate, and subjected to critical-point drying (CPD) at a pressure of 72.8 kg/cm^2 at –31ºC with liquid CO_2. The mucosal surface of each tissue was mounted on metal stubs, coated with gold thickness of approximately 20 nm. Finally, the tissues were scanned with a Hitachi S-530 Scanning Electron Microscope (SEM) at the University of Burdwan Scientific Instrument Center.

Histopathological fixation and staining: For histopathological study, the stomach, intestine, liver, and kidney tissues were fixed in Bouin's Fluid solution and then processed with graded alcohols. Blocks were prepared in 58–60ºC paraffin, and the thin sections of 3–4 μm were stained in Haematoxylin-Eosin.

RESULTS

ULTRASTRUCTURAL FINDINGS

Stomach: In the stomach, the most conspicuous ultrastructural alterations produced by NaF as seen by electron microscopy were the disarrangement and fragmentation of the mucosal folds, degeneration of epithelial cells (Figure 1), disintegration and loss of the microvilli from the apical plasma membrane of the epithelial cells, and excessive secretion of mucus from the epithelial cells (Figure 2). The scattered oriented gastric pits were surrounded by gastric epithelium cells, which also showed severe necrosis (Figure 2).

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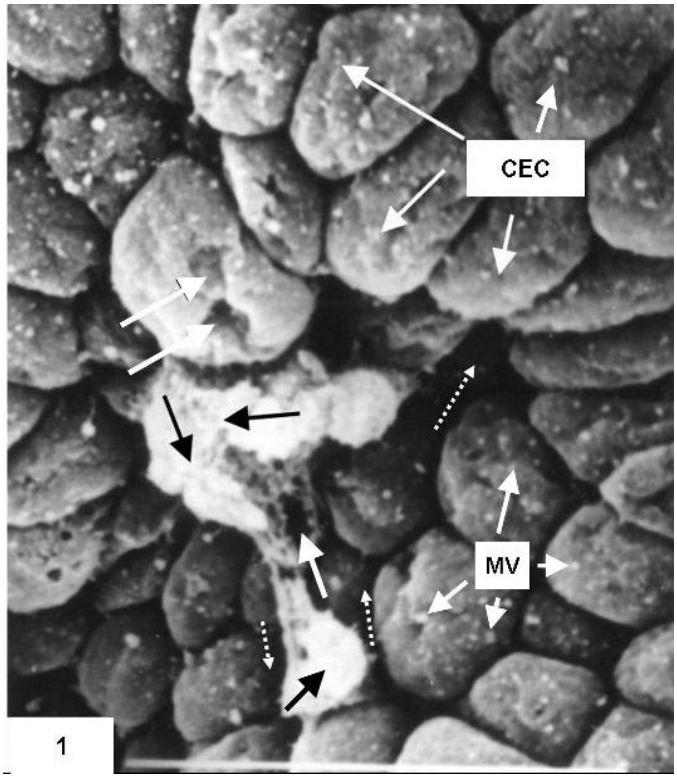

Figure 1. Ultramicroscopic photograph of NaF-treated fish stomach showing degenerated columnar epithelium cells (CEC) at the luminal surface (white arrow), loss of microvilli (MV), degeneration of epithelial cells (dotted white arrow), profuse secretion of mucus (black arrow). 3000 ×

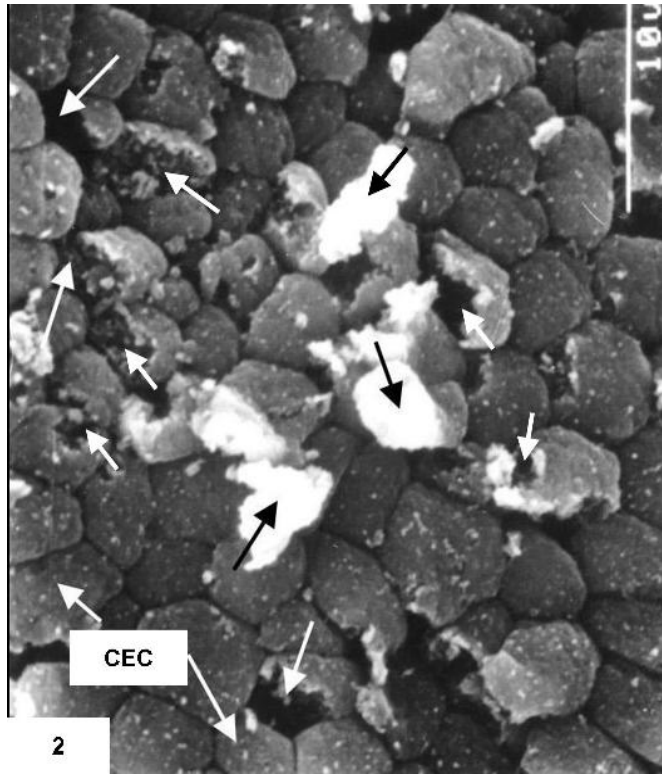

Figure 2. Ultramicroscopic photograph of NaF-treated fish stomach showing severe necrosis in CEC (white arrow), and excessive secretion of mucus (black arrow). 2500 ×

Intestine: Large areas of intestinal mucosal folds were damaged and débris of the fragmented secondary mucosal folds was observed in the cavities between the primary mucosal folds (Figure 3).

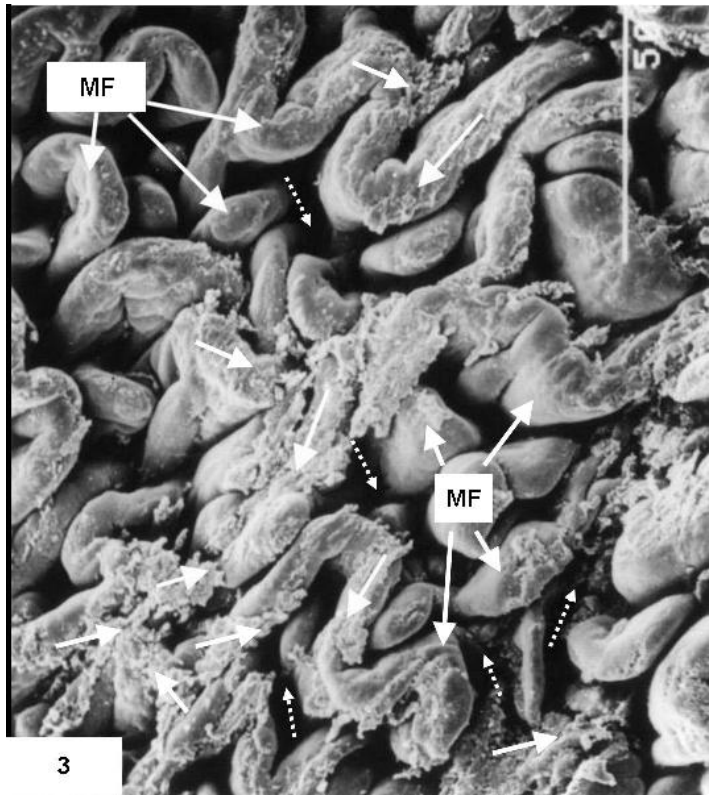

Figure 3.
Ultramicroscopic photograph of NaF-treated fish intestine showing the damaged mucosal folds (MF) (white arrow) and deterioration of secondary folds between the primary mucosal folds (dotted white arrow). 200 ×

The columnar epithelial cells showed the formation of even cell sheets leaving mucous cell openings and distortion of the boundaries of the epithelial cells followed by disintegration (Figures 4 and 5).

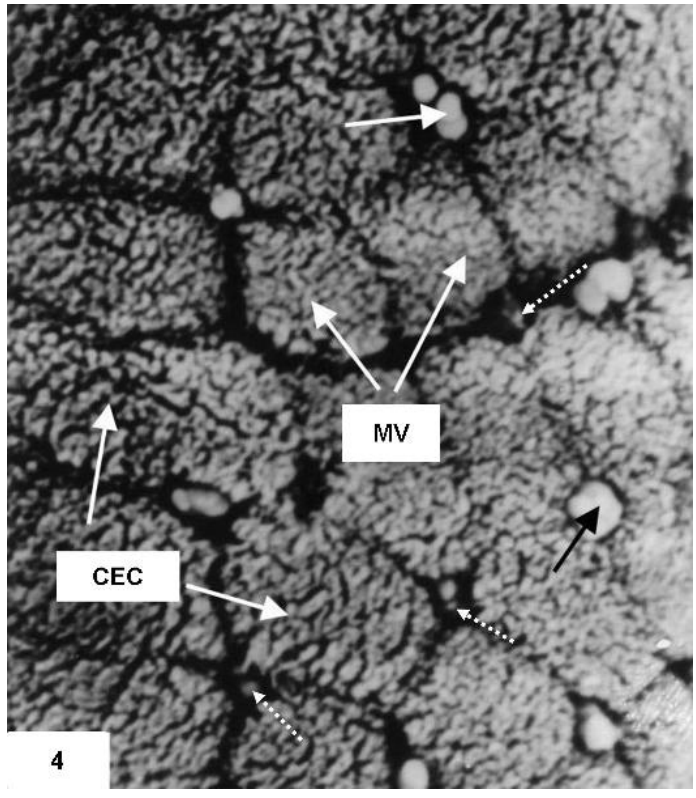

Figure 4.
Ultramicroscopic photograph of NaF-treated fish intestine showing distortion of cell boundaries of CEC (dotted white arrow) and degeneration of microvilli (MV). 5000 ×

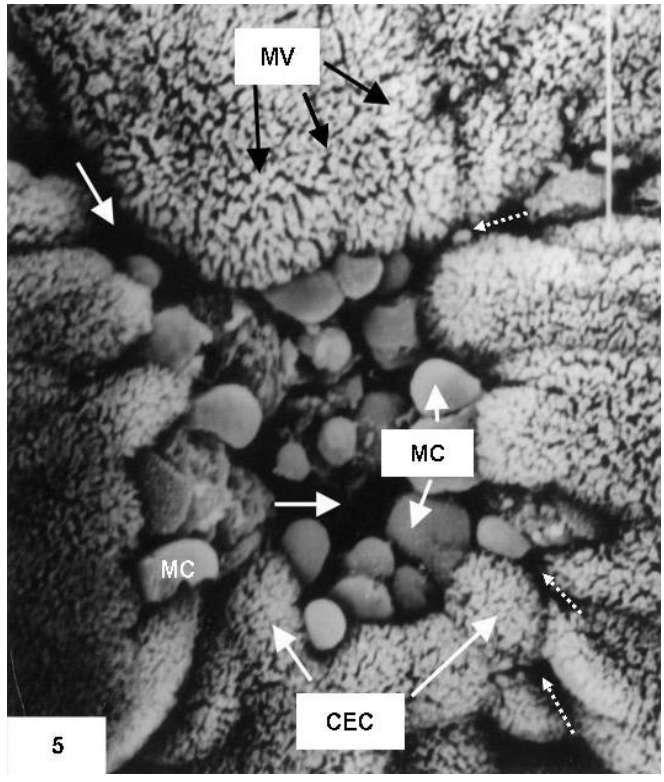

Figure 5.
Ultramicroscopic photograph of NaF-treated intestine showing distorted mucous cell (MC) opening surrounded by degenerated CEC and MV. 5000 ×

HISTOPATHOLOGICAL OBSERVATIONS

Stomach: Lesions detected by histopathology in the mucosal layer from NaF were very pronounced as compared to the control section (Figure 6).

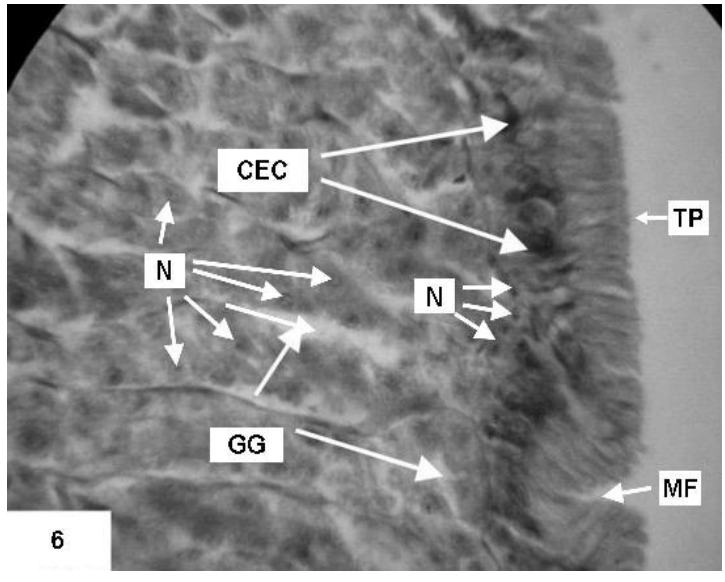

Figure 6.
Photomicrograph of stomach of control fish showing mucosal layer with prominent top plate (TP) and mucosal fold (MF). The CEC are lined beneath the mucosal layer and the tubular gastric gland (GG) with prominent nucleus (N). H & E staining. 10 × 40

Complete erosion in the top layer of mucous with severe vacuolation and degeneration in the gastric epithelium, along with disruption and necrosis of the gastric glands were also observed (Figure 7).

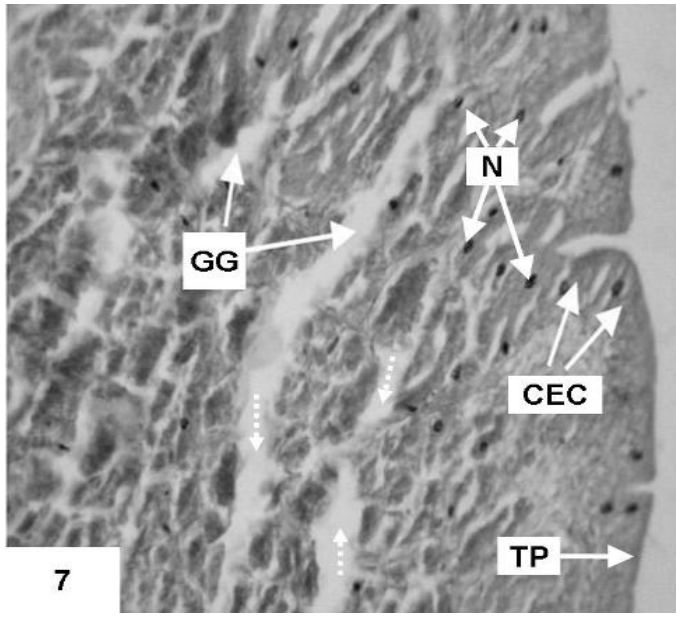

Figure 7. Stomach showing severe erosion in TP and MF. CEC and GG are showing extreme damage and vacuolation (dotted white arrow). H & E staining. 10 ×40

Intestine: Compared to the control (Figure 8), the F-exposed intestinal tissue showed degeneration of villi with severe necrosis in the absorptive columnar epithelial cells including brush border (Figure 9).

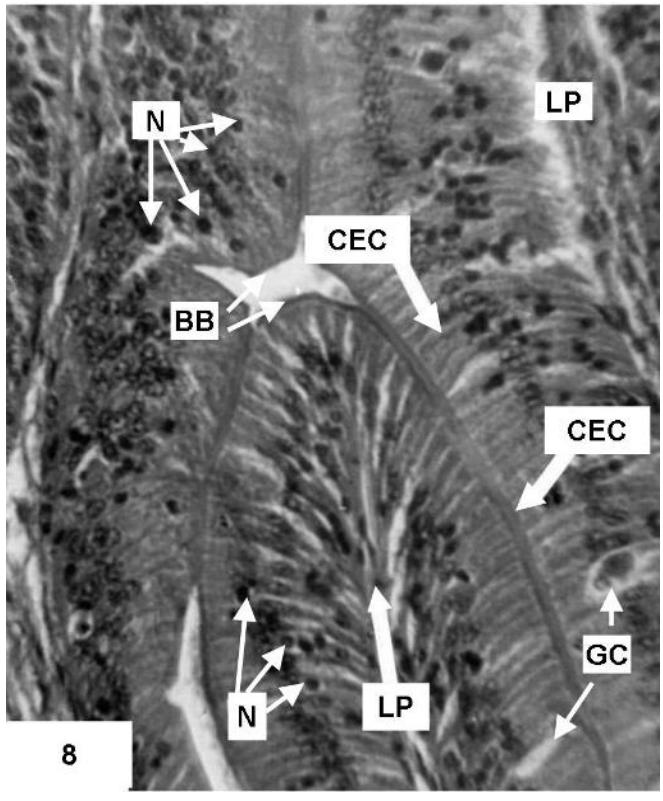

Figure 8. Photomicrograph of intestine of control fish showing prominent brush border (BB) in the apical region of the absorptive columnar epithelial cells (CEC) containing prominent nucleus. Presence of goblet cells (GC). H & E staining. 10 × 100

The nuclei of columnar epithelial cells exhibited pyknosis with fusion of boundaries of columnar epithelial cells (Figure 9). Degeneration of the submucosal layer, i.e., lamina propia and the absorptive columnar epithelial cells, resulted in vacuolation in the submucosal layer (Figure 9).

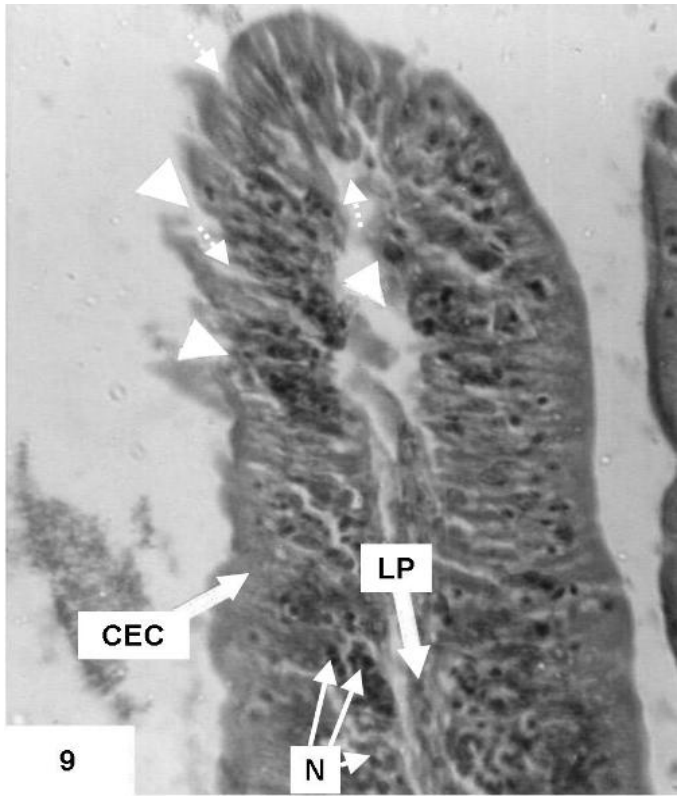

Figure 9.
Photomicrograph of NaF-treated fish intestine showing erosion of BB and LP (dotted white arrow). Necrosis of absorptive CEC is prominent. H & E staining. 10 × 40

Liver: The most conspicuous changes in the perilobular areas of the liver were the disarray of liver cord, damage to connective tissue, and degeneration of hepatocytes, in contrast to control tissue (Figure 10).

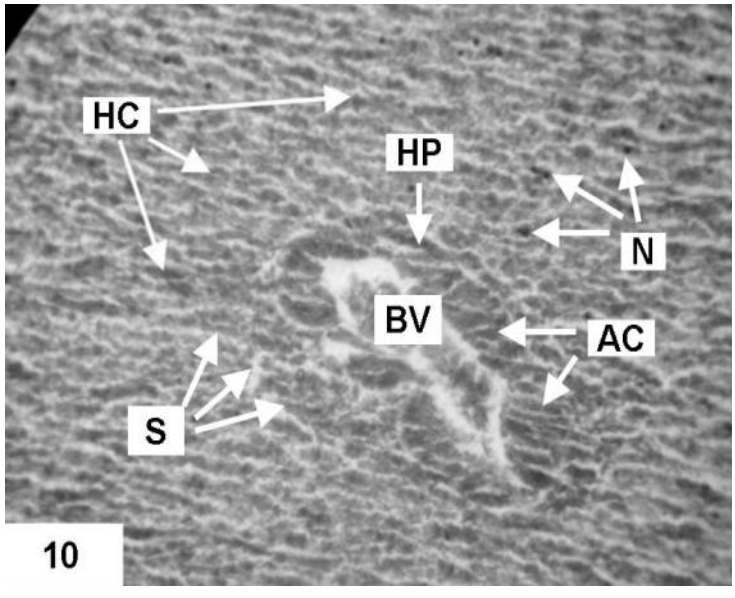

Figure 10.
Photomicrograph of liver of control fish showing chord-like arrangement of hepatocytes (HC). Prominent pancreatic acinar cells are also present. H & E staining. 10 × 10

The nuclei of the hepatocytes were enlarged and pyknotic (Figure 11).

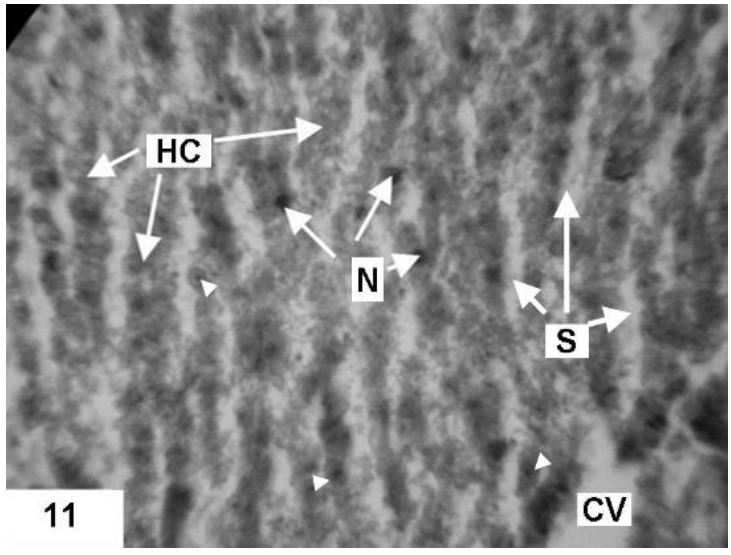

Figure 11. NaF treated fish liver hepatic cells (HC) and nuclei showing picnosis and enlarged nucleus (white arrow head); sinusoidal changes are very prominent (dotted white arrows). H & E staining. 10 × 40

Connective tissue was usually ruptured, but around the capillaries and venules it was slightly thickened. The capillary walls of the central vein were scarred. Inflammation was severe around central veins. The centrolobular area was more damaged than the perilobular area and showed focal necrosis (Figure 11). The cytoplasm of the hepatocytes was highly degenerated, and the cellular structure of hepatopancreas was altered. The nuclei were reduced in volume and had become pyknotic. The hepatocytes depicted a syncytial appearance with degenerated cytoplasm. In the surrounding blood vessels the pancreatic acinar cells and zymogen granules became scattered.

Kidney: In contrast to the control condition (Figure 12), disruption of the Bowman's capsule along with glomerular shrinkage, vacuolation occurred in the epithelial cell lining of the renal tubules with the enlarged nuclei showing a tendency to move away from the cells (Figure 13).

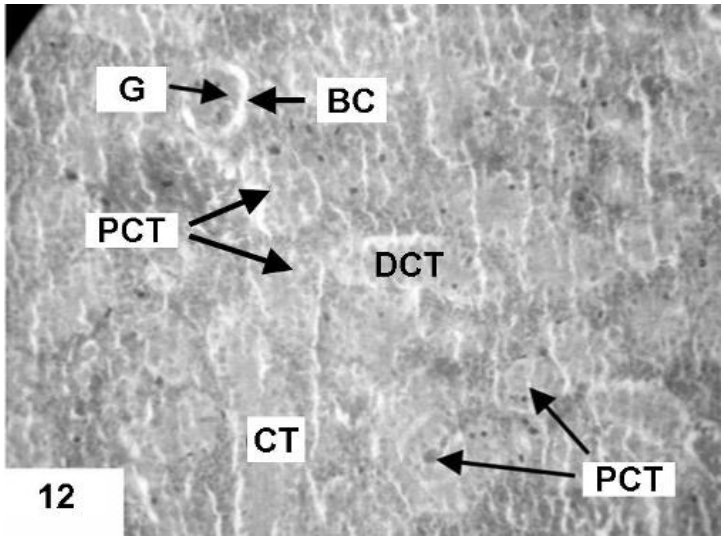

Figure 12. Photomicrograph of kidney of control fish showing regularly organised proximal convoluted tubules (PCT), distal convoluted tubules (DCT), collecting tubules (CT) and Bowman's capsule (BC). 10 ×40

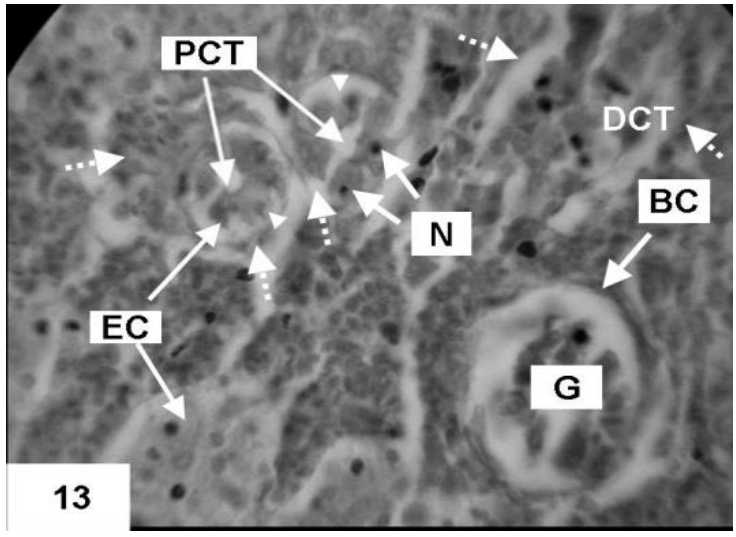

Figure 13. Histopathological microphotograph of NaF treated fish kidney showing glomerular (G) shrinkage, vacuolation in epithelial cell lining of proximal convoluted tubules (PCT). H & E staining. 10× 40

Histopathological damage in the different parts of the nephritic tissue caused enlargement in Bowman's capsules and destruction of the proximal and distal tubules. In the proximal tubule, intercellular partitions were lost, nuclei tended to move away, and the brush border became damaged. Vacuolation in the haematopoetic tissue was therefore clearly evident (Figure 13).

DISCUSSION

Earlier research indicated that F can pass through the cell membrane of soft tissue by simple diffusion and thereby cause impairment in soft tissue.[15,16] The complex nature of folding of the stomach wall allows for stretching during food consumption and also increases the surface area for digestive activity and longer retention of food items. The epithelial cells of the stomach have short, stubby microvilli that hold a neutral mucin film over gastric mucosa, thereby protecting the underlying epithelial cells from chemical injury.[17] The present study showed that F adversely affected the microridge structure of the gastric epithelium of the fish stomach, resulting in reduction of the protective ability of the gastric epithelium toward such injury and cell lysis. As a consequence, fragmentation in the mucous folds was observed. Scanning electron microscopy of the gastric mucosa has shown loss of microvilli, severely disrupted gastric pits, and loss of surface epithelium in fluorosis-affected patients.[9] Health surveys among the people affected by F through drinking water reveal a wide range of gastrointestinal disorders.[18] It is reasonable, therefore, to speculate from the present study that these types of damages from F intoxication may cause stomach distress of varying severity.

In the intestine, final digestion of ingested food and absorption takes place. Intestinal villi are the absorptive area of digested food. Our scanning electron microscope (SEM) study showed that the F toxicity in the intestine resulted in disruption of primary and secondary mucosal folds, formation of secondary mucosal-fold débris, distortion of the epithelial cell boundaries, and loss of microvilli. Histopathological observations revealed the fusion of the columnar epithelial cells at the basal region, degeneration of villi, pyknotic nuclei in

columnar epithelial cells, and ultimately severe necrosis in absorptive columnar epithelial cells including brush border. Widened junctions between adjacent epithelium cells, and a cracked clay-like appearance were found in duodenal mucosa of fluorosis patients.[9] Erosion and necrosis of surface mucosa, clumped submucosa, and hypertrophy of muscles in muscularis mucosa has been reported in the duodenum of NaF-treated rabbits.[10] Similar histopathological changes including flattening of villi, sloughing off of the mucosal lining, hypertrophy of epithelial cells, fusion of villi, rupture of villi tips, and cracked-clay appearance of the tissue in intestine were observed in F-treated *Labeo rohita*.[19] In the intestine of Swiss albino mice, hydropic degeneration in lamina propia of muscular tissue, increase in the number of goblet cells, broken tips of villi, nuclear pyknosis, and abnormal mitosis have been recorded from F toxicity.[11] All these reports are related to the present observations and confirm that F can cause serious abdominal distress.[18]

The primary metabolic organ by which detoxification of pollutants occurs is the liver. As a result of F toxicosis, disarray of liver cord, connective tissue damage, moderately vacuolated hepatocytes, degenerated cytoplasm, enlargement and pyknosis of hepatic nucleus, thickening and inflammation of central vein capillary wall, dilation of central vein and sinusoids with haemorrhage and engorged with blood, and ultimately focal necrosis were observed here in the treated fish. Similar observations including hepatocellular necrosis, degenerative changes, hepatic hyperplasia, vacuolization in hepatocytes, and centrolobular necrosis have been reported in the liver of rabbits from injection with NaF.[12] Zonal necrosis, pyknotic nucleus, and disarrangement of hepatic cords have been detected in rat liver after F intoxication.[20] As in the present study, vacuolar degeneration in the cytoplasm, loss of integrity in the epithelium lining of central vein, hepatocellular hypertrophy, hepatic sinusoidal dilation and enlargement, and distortion of parenchymatous cells at the lining of the central vein were observed in the liver of mice after the exposure to F in their drinking water. [13]

Finally, we consider the effects of F on the kidney, which is the primary organ for the excretion of toxic agents. Enlargement and disruption of the Bowman's capsule, glomerular shrinkage, vacuolation in the epithelial cells of the renal tubules, enlarged nuclei of the epithelial cells, enlargement of the lumen of the proximal tubules, loss of intercellular partitions, damage of the brush border in the proximal tubule, and vacuolation in the haematopoietic tissue were the evident in the kidney as a target organ for F toxicity to the fish. Thickening of Bowman's capsule, shrinkage of glomerulus, enhancement of capsular space, shrinkage of renal tubular lumen, and vacuolated cytoplasm of tubular epithelium cells were also observed in the kidney of *Labeo rohita* from F toxicosis.[19] Increasing amounts of cloudy swellings in the cell lining of the convoluted tubules, degeneration of tubular epithelia, tissue necrosis, vacuolization in renal tubules, hypertrophy and atrophy of glomeruli, exudation, interstitial oedema, and interstitial nephritis have been observed in rabbit kidney from F intoxication.[21,22] Atrophy of glomeruli, blood-filled spaces, and degeneration of tubular epithelium in kidney of mice treated with F in the drinking water were also recorded.[13] Therefore, the present study fully confirms kidney damage is caused by F intoxication.

In conclusion, we have shown that sub-lethal exposure of the teleost fish *Channa punctatus* to excess F causes significant disturbance and impairment of soft tissue organs involving the important life processes of digestion, metabolism, and excretion.

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